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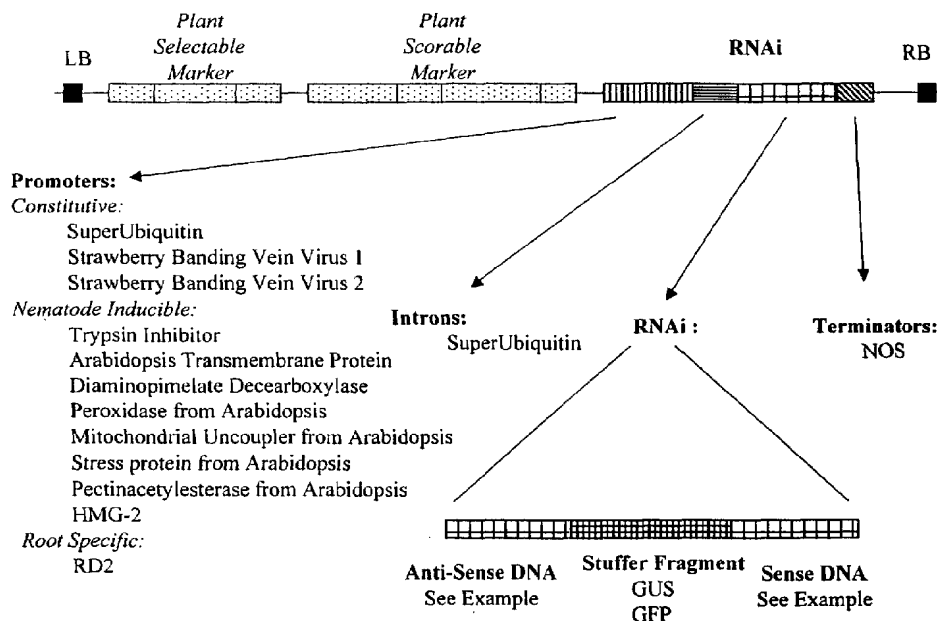
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[Continued on next page]

(54) Title: MATERIALS AND METHODS FOR THE CONTROL OF NEMATODES



(57) Abstract: The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides RNAi molecules, polynucleotide sequences, and methods of using these sequences in nematode control.



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## DESCRIPTION

### MATERIALS AND METHODS FOR THE CONTROL OF NEMATODES

#### Background of the Invention

[0001] Plant parasitic nematodes, such as root-knot nematodes (*Meloidogyne* species) and cyst nematodes (*Globodera* and *Heterodera*), attack nearly every food crop, and are among the world's most damaging agricultural pests. For example, root-knot nematodes parasitize more than 2,000 plant species from diverse plant families and represent a tremendous threat to crop production world-wide. These biotrophic pathogens have evolved highly specialized and complex feeding relationships with their hosts.

[0002] Nematodes cause millions of dollars of damage each year to turf grasses, ornamental plants, and food crops. Efforts to eliminate or minimize damage caused by nematodes in agricultural settings have typically involved the use of soil fumigation with materials such as chloropicrin, methyl bromide, and dazomet, which volatilize to spread the active ingredient throughout the soil. Such fumigation materials can be highly toxic and may create an environmental hazard. Various non-fumigant chemicals have also been used, but these too create serious environmental problems and can be highly toxic to humans.

[0003] Some research articles have been published concerning the effects of  $\delta$ -endotoxins from *B. thuringiensis* species on the viability of nematodes. See, for example, Bottjer, Bone and Gill ([1985] *Experimental Parasitology* 60:239-244); Ignoffo and Dropkin (Ignoffo, C.M., Dropkin, V.H. [1977] *J. Kans. Entomol. Soc.* 50:394-398); and Ciordia, H. and W.E. Bizzell ([1961] *Jour. of Parasitology* 47:41 [abstract]). Several patents have issued describing the control of nematodes with *B.t.* See, for example, U.S. Patent Nos. 4,948,734; 5,093,120; 5,281,530; 5,426,049; 5,439,881; 5,236,843; 5,322,932; 5,151,363; 5,270,448; 5,350,577; 5,667,993; and 5,670,365. The development of resistance by insects to *B.t.* toxins is one obstacle to the successful use of such toxins.

[0004] The pesticidal activity of avermectins is well known. The avermectins are disaccharide derivatives of pentacyclic, 16-membered lactones. They can be divided into four major compounds: A<sub>1a</sub>, A<sub>2a</sub>, B<sub>1a</sub>, and B<sub>2a</sub>; and four minor compounds: A<sub>1b</sub>, A<sub>2b</sub>, B<sub>1b</sub>, and B<sub>2b</sub>. The isolation and purification of these compounds is also described in U.S. Patent No. 4,310,519, issued January 12, 1982. Avermectin B<sub>2a</sub> is active against the root-knot nematode, *Meloidogyne incognita*. It is reported to be 10-30 times as potent as commercial contact nematicides when incorporated into soil at 0.16-0.25 kg/ha (Boyce Thompson Institute for Plant Research 58th Annual Report [1981]; Putter, I. *et al.* [1981] "Avermectins: Novel Insecticides, Acaracides, and Nematicides from a Soil Microorganism," *Experientia* 37:963-964). Avermectin B<sub>2a</sub> is not toxic to tomatoes or cucumbers at rates of up to 10 kg/ha.

[0005] Fatty acids are a class of natural compounds which occur abundantly in nature and which have interesting and valuable biological activities. Tarjan and Cheo (Tarjan, A.C., P.C. Cheo [1956] "Nematocidal Value of Some Fatty Acids," Bulletin 332, Contribution 884, Agricultural Experiment Station, University of Rhode Island, Kingston, 41 pp.) report the activity of certain fatty acids against nematodes. In 1977 Sitaramaiah and Singh (Sitaramaiah, K., R.S. Singh [1977] *Indian J. Nematol.* 7:58-65) also examined the response of nematodes to fatty acids. The results of these tests with short chain acids were equivocal, showing nematode-inhibitory action in some instances and stimulatory activity in other instances. Phytotoxicity of these acids was observed at higher concentrations. The short chain fatty acids were also examined by Malik and Jairajpuri (Malik, Z., M.S. Jairajpuri [1977] *Nematol. medit.* 12:73-79), who observed nematode toxicity at high concentrations of the fatty acids.

[0006] Notwithstanding the foregoing (some of the limitations of and problems associated with these approaches are discussed above), there is a need for safe and effective alternatives for controlling nematodes.

[0007] One method for disrupting normal cellular processes is by the use double-stranded interfering RNA (RNAi), or RNA-mediated interference (RNAi). When RNAi corresponding to a sense and antisense sequence of a target mRNA is introduced into a cell, the targeted mRNA is degraded and protein translation of that message is stopped. Although not yet fully understood, the mechanism of this post-transcriptional gene

silencing appears to be at least partially due to the generation of small RNA molecules, about 21 - 25 nucleotides in length, that correspond to the sense and antisense pieces of the RNAi introduced into the cell (Bass, B. L. [2000] "Double-stranded RNA as a template for gene silencing" *Cell* 101:235-238).

[0008] The specificity of this gene silencing mechanism appears to be extremely high, blocking expression only of targeted genes, while leaving other genes unaffected. A recent example of the use of RNAi; to inhibit genetic function in plants used *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* (Chuang, C.-F. and E. M. Meyerowitz [2000] "Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*" *Proc. Natl. Acad. Sci. USA* 97:4985-4990). Chuang *et al.* describe the construction of vectors delivering variable levels of RNAi targeted to each of four genes involved in floral development. Severity of abnormal flower development varied between transgenic lines. For one of the genes, AGAMOUS (AG), a strong correlation existed between declining accumulation of mRNA and increasingly severe phenotypes, suggesting that AG-specific endogenous mRNA is the target of RNAi.

#### Brief Summary of the Invention

[0009] The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides polynucleotide sequences that encode nematode genes, RNAi that selectively targets mRNA transcripts of these essential nematode genes, and methods of using these sequences in nematode control strategies. Such sequences for use according to the subject invention are summarized in Appendix 1. RNAi molecules disclosed herein can be used to inhibit the expression of one or more of these genes in nematodes.

### Brief Description of the Drawings

[00010] **Figure 1:** Modular Binary Construct System (MBCS): A series of six, 8-base cutter restriction enzyme sites has been placed between the left and right Ti borders of a previously created kan<sup>R</sup>/tet<sup>R</sup> binary plasmid.

[00011] **Figure 2:** An exemplary shuttle vector created for cloning of useful DNA fragments by containing the multi-cloning site (MCS) of a modified Bluescript plasmid flanked by 8-base restriction sites.

[00012] **Figure 3:** An exemplary shuttle vector with exemplary inserts.

[00013] **Figure 4:** A suggested RNAi binary vector with exemplary inserts.

[00014] **Figure 5:** Exemplary selectable markers for MBCS.

[00015] **Figure 6:** Exemplary scorable markers for MCBS.

[00016] **Figure 7:** Exemplary RNAi binary vector.

[00017] **Figure 8:** Exemplary RNAi shuttle vector.

### Brief Description of the Sequences

[00018] Brief Description of the Sequences can be found in Appendix I.

### Detailed Disclosure of the Invention

[00019] The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides polynucleotide sequences and methods of using these sequences in nematode control strategies. A preferred method for controlling nematodes according to the subject invention provides materials and methods for controlling nematodes by using double-stranded interfering RNA (RNAi), or RNA-mediated interference (RNAi). The terms RNAi and RNAi are used interchangeably herein unless otherwise noted.

[00020] In one embodiment of the invention, RNAi molecules are provided which are useful in methods of killing nematodes and/or inhibiting their growth, development, parasitism or reproduction. RNAi molecules of the invention are also useful for the regulation of levels of specific mRNA in nematodes.

[00021] dsRNA (RNAi) typically comprises a polynucleotide sequence identical to a target gene (or fragment thereof) linked directly, or indirectly, to a polynucleotide

sequence complementary to the sequence of the target gene (or fragment thereof). The dsRNA may comprise a polynucleotide linker (stuffer) sequence of sufficient length to allow for the two polynucleotide sequences to fold over and hybridize to each other; however, a linker sequence is not necessary. The linker (stuffer) sequence is designed to separate the antisense and sense strands of RNAi significantly enough to limit the effects of steric hindrances and allow for the formation of dsRNA molecules.

[00022] RNA containing a nucleotide sequence identical to a fragment of the target gene is preferred for inhibition; however, RNA sequences with insertions, deletions, and point mutations relative to the target sequence can also be used for inhibition. Sequence identity may be optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, *Sequence Analysis Primer*, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a fragment of the target gene transcript.

[00023] As disclosed herein, 100% sequence identity between the RNA and the target gene is not required to practice the present invention. Thus the invention has the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence.

[00024] RNA may be synthesized either *in vivo* or *in vitro*. Endogenous RNA polymerase of the cell may mediate transcription *in vivo*, or cloned RNA polymerase can be used for transcription *in vivo* or *in vitro*. For transcription from a transgene *in vivo* or an expression construct, a regulatory region (e.g., promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the RNA strand (or strands). Inhibition may be targeted by specific transcription in an organ, tissue, or cell type; stimulation of an environmental condition (e.g., infection, stress, temperature, chemical inducers); and/or engineering transcription at a developmental stage or age. The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus. RNA

may be chemically or enzymatically synthesized by manual or automated reactions. The RNA may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (e.g., T3, T7, SP6). The use and production of an expression construct are known in the art (see, for example, WO 97/32016; U.S. Pat. Nos. 5,593,874; 5,698,425; 5,712,135; 5,789,214; and 5,804,693; and the references cited therein). If synthesized chemically or by *in vitro* enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the RNA may be used with no or a minimum of purification to avoid losses due to sample processing. The RNA may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

[00025] Preferably and most conveniently, RNAi can be targeted to an entire polynucleotide sequence of a gene set forth herein. Preferred RNAi molecules of the instant invention are highly homologous or identical to the polynucleotides summarized in Appendix 1. The homology is preferably greater than 90% and is most preferably greater than 95%.

[00026] Fragments of genes can also be targeted. These fragments are typically in the approximate size range of about 20 nucleotides. Thus, targeted fragments are preferably at least about 15 nucleotides. In certain embodiments, the gene fragment targeted by the RNAi molecule is about 20-25 nucleotides in length. However, other size ranges can also be used. For example, using a *C. elegans* microinjection assay, RNAi "fragments" of about 60 nucleotides with between 95 and 100% identity (to a nematode gene) were determined to cause excellent inhibition.

[00027] Thus, RNAi molecules of the subject invention are not limited to those that are targeted to the full-length polynucleotide or gene. The nematode gene product can be inhibited with a RNAi molecule that is targeted to a portion or fragment of the exemplified polynucleotides; high homology (90-95%) or identity is also preferred, but not necessarily essential, for such applications.

[00028] The polynucleotide sequences identified in Appendix A and shown in the Sequence ID listing are from genes encoding nematode proteins having the functions



shown in Appendix 1. The genes exemplified herein are representative of particular classes of proteins which are preferred targets for disruption according to the subject invention. These classes of proteins include, for example, proteins involved in ribosome assembly; neuro transmitter receptors and ligands; electron transport proteins; metabolic pathway proteins; and protein and polynucleotide production, folding, and processing proteins.

[00029] Genetic regulatory sequences, such as promoters, enhancers, and terminators, can be used in genetic constructs to practice the subject invention. Such constructs themselves can also be used for nematode control. Various constructs can be used to achieve expression in specific plant tissues (by using root specific promoters, for example) and/or to target specific nematode tissues (by using targeting elements or adjacent targeting sequences, for example).

[00030] In a specific embodiment of the subject invention, plant cells, preferably root cells, are genetically modified to produce at least one RNAi that is designed to be taken up by nematodes during feeding to block expression (or the function of) of a target gene. As is known in the art, RNAi can target and reduce (and, in some cases, prevent) the translation of a specific gene product. RNAi can be used to reduce or prevent message translation in any tissue of the nematode because of its ability to cross tissue and cellular boundaries. Thus, RNAi that is contacted with a nematode by soaking, injection, or consumption of a food source will cross tissue and cellular boundaries. RNAi can also be used as an epigenetic factor to prevent the proliferation of subsequent generations of nematodes.

[00031] Nematode polynucleotide sequences disclosed herein demonstrate conserved nucleotide motifs among different nematode genera. Conserved nucleotide motifs strongly suggest that these sequences are associated with viability and/or parasitism and are functionally conserved and expressed in both *Meloidogyne incognita* (root-knot nematode) and *Globodera rostochiensis* and *Globodera pallids* (potato cyst nematodes). The use of these polynucleotides, and RNAi inhibitors thereof, is advantageous because such RNAi can be designed to have broad RNAi specificity and are thus useful for controlling a large number of plant parasitic nematodes *in planta*. Because the genes identified in this disclosure are associated with nematode survival

and/or parasitism, RNAi inhibition of these genes (arising from contacting nematodes with compositions comprising RNAi molecules) prevents and/or reduces parasitic nematode growth, development, and or parasitism.

[00032] Methods of the subject invention include the transformation of plant cells with genes or polynucleotides of the present invention, which can be used to produce nematode inhibitors or RNAi in the plants. In one embodiment, the transformed plant or plant tissue can express RNAi molecules encoded by the gene or polynucleotide sequence introduced into the plant. Other nematode inhibitors contemplated by the invention include antisense molecules specific to the polynucleotide sequences disclosed herein. The transformation of plants with genetic constructs disclosed herein can be accomplished using techniques well known to those skilled in the art and can involve modification of the gene(s) to optimize expression in the plant to be made resistant to nematode infection and infestation. Furthermore, it is known in the art that many tissues of the transgenic plants (such as the roots) can be targeted for transformation.

[00033] RNA-mediated interference (RNAi) of gene expression. Several aspects of root-knot nematode biology make classical genetic studies difficult with this organism. Since root-knot nematodes reproduce by obligatory mitotic parthenogenesis, the opportunity to perform genetic crosses is not available. Microinjection of RNAi can be used to manipulate gene expression in *C. elegans* (Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. [1998] "Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*" *Nature* 391:806- 811). Microinjecting (into adult nematodes) RNAi can turn off specific genes in progeny worms complementary to the coding region of the genes. Moreover, gene inhibition occurs in progeny when RNAi is injected into the body cavity of the adult, indicating the ability of the RNAi to cross cellular boundaries. This RNAi injection method provides a molecular genetic tool that allows for analysis of gene function in root-knot nematodes.

[00034] RNAi can be taken up by *C. elegans* by simply soaking the nematodes in a solution RNAi. This results in targeted inhibition of gene expression in the nematode (Maeda, I., Y. Kohara, M. Yamamoto and A. Sugimoto [1999] "RNAi screening with a non-redundant cDNA set" International Worm Meeting, Madison, WI, abstract 565). Nematodes fed *E. coli* expressing RNAi also demonstrate targeted and

heritable inhibition of gene expression (Sarkissian, M., H. Tabara and C. C. Mello [1999] "A mut-6 screen for RNAi deficient mutants" International Worm Meeting, Madison, WI, abstract 741; Timmons, I. and A. Fire [1998] "Specific interference by ingested dsRNA" *Nature* 395:854; WO 99/32619, hereby incorporated by reference in its entirety).

[00035] Accordingly, one aspect of the instant invention is directed to the control of nematodes comprising contacting nematodes with compositions comprising RNAi molecules specific to the nematode genes disclosed herein. The contacting step may include soaking the nematodes in a solution containing RNAi molecules, feeding nematodes RNAi molecules contained in microbes or plant cells upon which the nematode feeds, or injecting nematodes with RNAi. Nematodes can also be "contacted" and controlled by RNAi expressed in plant tissues that would be consumed, ingested, or frequented by nematodes.

[00036] The RNAi molecules provided to the nematodes may be specific to a single gene. A "cocktail" of RNAi molecules specific to various segments of a single gene can also be used. In addition, a "multigene cocktail" of RNAi molecules specific to two or more genes (or segments thereof) may be applied to the nematodes according to the subject invention.

[00037] In addition to RNAi uptake mediated by transgenic plants, nematodes can be directly transformed with RNAi constructs of cDNAs encoding secretory or other essential proteins to reduce expression of the corresponding gene. The transgenic animals can be assayed for inhibition of gene product using immunoassays or for reduced virulence on a host. Progeny of affected worms can also be assayed by similar methods.

[00038] Procedures that can be used for the preparation and injection of RNAi include those detailed by Fire *et al.*, (1998; <ftp://ciw1.ciwemb.edu>). Root-knot nematodes can be routinely monoxenically cultured on *Arabidopsis thaliana* roots growing on Gamborg's B-5/Gelrite® media. This nematode-host pathosystem is ideally suited for these microinjection experiments since limited root galling results in the parasitic stages (late J2 through adult females) developing outside of the root for easy accessibility for injecting. Another advantage is the parthenogenic reproduction of root-knot nematodes, which makes fertilization by males unnecessary for egg production. The RNAi can be injected into the body cavity of parasitic stages of root-knot nematodes

feeding on *A. thaliana* roots using microinjection. Control nematodes can be injected in parallel with only buffer or an unrelated RNAi. Injected nematodes can be monitored for egg production, and the eggs can be collected for the assays described below. Female root-knot nematodes will typically survive and lay more than 250 eggs following 1  $\mu$ l injection of buffer.

[00039] Alternatively, methods are available for microinjecting materials directly into the plant root cells upon which nematodes feed: giant cells or syncytial cells (Böckenhoff, A. and F.M.W. Grundler [1994] "Studies on the nutrient uptake by the beet cyst nematode *Heterodera schachtii* by *in situ* microinjection of fluorescent probes into the feeding structures in *Arabidopsis thaliana*" *Parasitology* 109:249-254). This provides an excellent test system to screen RNAi molecules for efficacy by directly inhibiting growth and development of the nematode feeding upon the microinjected plant cell, or by reducing fecundity and the ability of said nematode to generate pathogenic or viable progeny.

[00040] There are a number of strategies that can be followed to assay for RNAi gene interference. Inhibition of gene expression by RNAi inhibits the accumulation of the corresponding secretory protein in the esophageal gland cells of transgenic J2 hatched from the eggs produced by the injected nematodes. In the first assay, polyclonal antibodies to the target gene product can be used in immunolocalization studies (Hussey, R. S. [1989] "Monoclonal antibodies to secretory granules in esophageal glands of *Meloidogyne* species" *J. Nematol.* 21:392-398; Borgonie, G, E. van Driessche, C. D. Link, D. de Waele, and A. Coomans [1994] "Tissue treatment for whole mount internal lectin staining in the nematodes *Caenorhabditis elegans*, *Panagrolaimus superbus* and *Acrobeloides maximus*" *Histochemistry* 101:379-384) to monitor the synthesis of the target protein in the gland cells of progeny of the injected nematodes, or in any other nematode tissue that fails to express the essential targeted gene. Interference of endogenous gene activity by the RNAi eliminates binding of the antibodies to secretory granules in the glands, or any other target tissue, of the transgenic nematodes, and can be monitored by these *in situ* hybridization experiments. Control nematodes injected only with the injection buffer can be processed similar to the RNAi treated nematodes.

[00041] Another assay is designed to determine the effect of the RNAi on reducing the virulence of J2 progeny of the injected females. Egg masses from injected females can be transferred singly to *A. thaliana* plates to assess the ability of the transgenic J2 to infect roots. The J2 hatching from the eggs transferred to the plates can be monitored; after 25 days the number of galls with egg laying females can be recorded. The *A. thaliana* roots can also be stained with acid fuchsin to enumerate the number of nematodes in the roots. Egg masses from nematodes injected only with the injection buffer can be handled similarly and used as controls. The treatments can be replicated, and the root infection data can be analyzed statistically. These experiments can be used to assess the importance of the target genes in root-knot nematode's virulence or viability. By staining the J2 progeny of the injected females with the antibodies, it can be determined whether RNAi blocks expression of the targeted gene.

[00042] Additional uses of polynucleotides. The polynucleotide sequences exemplified herein can be used in a variety of ways. These polynucleotides can be used in assays for additional polynucleotides and additional homologous genes, and can be used in tracking the quantitative and temporal expression of parasitism genes in nematodes. These polynucleotides can be cloned into microbes for production and isolation of their gene products. Among the many uses of the isolated gene product is the development of additional inhibitors and modifiers. The protein products of the subject polynucleotides can also be used as diagnostic tools. For example, proteins encoded by the parasitism genes, as identified herein, can be used in large scale screenings for additional peptide inhibitors. The use of peptide phage display screening is one method that can be used in this regard. Thus, the subject invention also provides new biotechnological strategies for managing nematodes under sustainable agricultural conditions.

[00043] Antisense technologies can also be used for phytopathogenic nematode control. Antisense technology can be used to interfere with expression of the disclosed endogenous nematode genes. Antisense technology can also be used to alter the components of plants used as targets by the nematodes. For example, the transformation of a plant with the reverse complement of an endogenous gene encoded by a polynucleotide exemplified herein can result in strand co-suppression and gene silencing

or inhibition of a target involved in the nematode infection process. Thus, the subject invention includes transgenic plants (which are preferably made nematode-resistant in this manner, and other organisms including microbes and phages) comprising RNAi or antisense molecules specific to any of the polynucleotides identified herein.

[00044] Polynucleotide probes. DNA possesses a fundamental property called base complementarity. In nature, DNA ordinarily exists in the form of pairs of anti-parallel strands, the bases on each strand projecting from that strand toward the opposite strand. The base adenine (A) on one strand will always be opposed to the base thymine (T) on the other strand, and the base guanine (G) will be opposed to the base cytosine (C). The bases are held in apposition by their ability to hydrogen bond in this specific way. Though each individual bond is relatively weak, the net effect of many adjacent hydrogen bonded bases, together with base stacking effects, is a stable joining of the two complementary strands. These bonds can be broken by treatments such as high pH or high temperature, and these conditions result in the dissociation, or "denaturation," of the two strands. If the DNA is then placed in conditions which make hydrogen bonding of the bases thermodynamically favorable, the DNA strands will anneal, or "hybridize," and reform the original double-stranded DNA. If carried out under appropriate conditions, this hybridization can be highly specific. That is, only strands with a high degree of base complementarity will be able to form stable double-stranded structures. The relationship of the specificity of hybridization to reaction conditions is well known. Thus, hybridization may be used to test whether two pieces of DNA are complementary in their base sequences. It is this hybridization mechanism which facilitates the use of probes of the subject invention to readily detect and characterize DNA sequences of interest.

[00045] The specifically exemplified polynucleotides of the subject invention can themselves be used as probes. Additional polynucleotide sequences can be added to the ends of (or internally in) the exemplified polynucleotide sequences so that polynucleotides that are longer than the exemplified polynucleotides can also be used as probes. Thus, isolated polynucleotides comprising one or more of the exemplified sequences are within the scope of the subject invention. Polynucleotides that have less nucleotides than the exemplified polynucleotides can also be used and are contemplated within the scope of the present invention. For example, for some purposes, it might be

useful to use a conserved sequence from an exemplified polynucleotide wherein the conserved sequence comprises a portion of an exemplified sequence. Thus, polynucleotides of the subject invention can be used to find additional, homologous (wholly or partially) genes.

[00046] Probes of the subject invention may be composed of DNA, RNA, or PNA (peptide nucleic acid). The probe will normally have at least about 10 bases, more usually at least about 17 bases, and may have about 100 bases or more. Longer probes can readily be utilized, and such probes can be, for example, several kilobases in length. The probe sequence is designed to be at least substantially complementary to a portion of a gene encoding a protein of interest. The probe need not have perfect complementarity to the sequence to which it hybridizes. The probes may be labeled utilizing techniques that are well known to those skilled in this art.

[00047] One approach for the use of the subject invention as probes entails first identifying DNA segments that are homologous with the disclosed nucleotide sequences using, for example, Southern blot analysis of a gene bank. Thus, it is possible, without the aid of biological analysis, to know in advance the probable activity of many new polynucleotides, and of the individual gene products expressed by a given polynucleotide. Such an analysis provides a rapid method for identifying commercially valuable compositions.

[00048] One hybridization procedure useful according to the subject invention typically includes the initial steps of isolating the DNA sample of interest and purifying it chemically. Either lysed nematodes or total fractionated nucleic acid isolated from nematodes can be used. Cells can be treated using known techniques to liberate their DNA (and/or RNA). The DNA sample can be cut into pieces with an appropriate restriction enzyme. The pieces can be separated by size through electrophoresis in a gel, usually agarose or acrylamide. The pieces of interest can be transferred to an immobilizing membrane.

[00049] The particular hybridization technique is not essential to the subject invention. As improvements are made in hybridization techniques, they can be readily applied.

[00050] The probe and sample can then be combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs. Thereafter, the membrane is washed free of extraneous materials, leaving the sample and bound probe molecules typically detected and quantified by autoradiography and/or liquid scintillation counting. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong non-covalent bond between the two molecules, it can be reasonably assumed that the probe and sample are essentially identical or very similar. The probe's detectable label provides a means for determining in a known manner whether hybridization has occurred.

[00051] In the use of the nucleotide segments as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include  $^{32}\text{P}$ ,  $^{35}\text{S}$ , or the like. Non-radioactive labels include, for example, ligands such as biotin or thyroxine, as well as enzymes such as hydrolases or peroxidases, or the various chemiluminescers such as luciferin, or fluorescent compounds like fluorescein and its derivatives. In addition, the probes can be made inherently fluorescent as described in International Application No. WO 93/16094.

[00052] Various degrees of stringency of hybridization can be employed. The more stringent the conditions, the greater the complementarity that is required for duplex formation. Stringency can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*, Stockton Press, New York, NY., pp. 169-170.

[00053] As used herein "moderate to high stringency" conditions for hybridization refers to conditions that achieve the same, or about the same, degree of specificity of hybridization as the conditions "as described herein." Examples of moderate to high stringency conditions are provided herein. Specifically, hybridization of immobilized DNA on Southern blots with  $^{32}\text{P}$ -labeled gene-specific probes was performed using standard methods (Maniatis *et al.*). In general, hybridization and subsequent washes were carried out under moderate to high stringency conditions that



allowed for detection of target sequences with homology to sequences exemplified herein. For double-stranded DNA gene probes, hybridization was carried out overnight at 20-25° C below the melting temperature ( $T_m$ ) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula from Beltz *et al.* (1983):

[00054]  $T_m = 81.5^\circ\text{C} + 16.6 \log[\text{Na}^+] + 0.41(\%G+C) - 0.61(\%\text{formamide}) - 600/\text{length of duplex in base pairs.}$

Washes are typically carried out as follows:

- (1) Twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash).
- (2) Once at  $T_m - 20^\circ\text{C}$  for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

[00055] For oligonucleotide probes, hybridization was carried out overnight at 10-20° C below the melting temperature ( $T_m$ ) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA.  $T_m$  for oligonucleotide probes was determined by the following formula from Suggs *et al.* (1981):

[00056]  $T_m (^\circ\text{C}) = 2(\text{number T/A base pairs}) + 4(\text{number G/C base pairs})$

[00057] Washes were typically carried out as follows:

- [00058] (1) Twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash).
- [00059] (2) Once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

[00060] In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment of greater than about 70 or so bases in length, the following conditions can be used:

- |           |                                |
|-----------|--------------------------------|
| Low:      | 1 or 2X SSPE, room temperature |
| Low:      | 1 or 2X SSPE, 42° C            |
| Moderate: | 0.2X or 1X SSPE, 65° C         |
| High:     | 0.1X SSPE, 65° C.              |

[00061] Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch

can be tolerated. Therefore, polynucleotide sequences of the subject invention include mutations (both single and multiple), deletions, and insertions in the described sequences, and combinations thereof, wherein said mutations, insertions, and deletions permit formation of stable hybrids with a target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence using standard methods known in the art. Other methods may become known in the future.

[00062] The mutational, insertional, and deletional variants of the polynucleotide sequences of the invention can be used in the same manner as the exemplified polynucleotide sequences so long as the variants have substantial sequence similarity with the original sequence. As used herein, substantial sequence similarity refers to the extent of nucleotide similarity that is sufficient to enable the variant polynucleotide to function in the same capacity as the original sequence. Preferably, this similarity is greater than 50%; more preferably, this similarity is greater than 75%; and most preferably, this similarity is greater than 90%. The degree of similarity needed for the variant to function in its intended capacity will depend upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations that are designed to improve the function of the sequence or otherwise provide a methodological advantage.

[00063] PCR technology. Polymerase Chain Reaction (PCR) is a repetitive, enzymatic, primed synthesis of a nucleic acid sequence. This procedure is well known and commonly used by those skilled in this art (see U.S. Patent Nos. 4,683,195; 4,683,202; and 4,800,159; Saiki *et al.*, 1985). PCR is based on the enzymatic amplification of a DNA fragment of interest that is flanked by two oligonucleotide primers that hybridize to opposite strands of the target sequence. The primers are oriented with the 3' ends pointing towards each other. Repeated cycles of heat denaturation of the template, annealing of the primers to their complementary sequences, and extension of the annealed primers with a DNA polymerase result in the amplification of the segment defined by the 5' ends of the PCR primers. Since the extension product of each primer can serve as a template for the other primer, each cycle essentially doubles the amount of DNA fragment produced in the previous cycle. This results in the exponential accumulation of the specific target fragment, up to several million-fold in a

few hours. By using a thermostable DNA polymerase such as *Taq* polymerase, which is isolated from the thermophilic bacterium *Thermus aquaticus*, the amplification process can be completely automated. Other enzymes that can be used are known to those skilled in the art.

[00064] The polynucleotide sequences of the subject invention (and portions thereof such as conserved regions and portions that serve to distinguish these sequences from previously-known sequences) can be used as, and/or used in the design of, primers for PCR amplification. In performing PCR amplification, a certain degree of mismatch can be tolerated between primer and template. Therefore, mutations, deletions, and insertions (especially additions of nucleotides to the 5' end) of the exemplified polynucleotides can be used in this manner. Mutations, insertions and deletions can be produced in a given primer by methods known to an ordinarily skilled artisan.

[00065] The polynucleotide sequences of the instant invention may be "operably linked" to regulatory sequences such as promoters and enhancers. Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is "operably linked" to DNA encoding a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is "operably linked" to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is "operably linked" to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[00066] Polynucleotides and proteins. Polynucleotides of the subject invention can be defined according to several parameters. One characteristic is the biological activity of the protein products as identified herein. The proteins and genes of the subject invention can be further defined by their amino acid and nucleotide sequences. The sequences of the molecules can be defined in terms of homology to certain exemplified sequences as well as in terms of the ability to hybridize with, or be amplified by, certain

exemplified probes and primers. Additional primers and probes can readily be constructed by those skilled in the art such that alternate polynucleotide sequences encoding the same amino acid sequences can be used to identify and/or characterize additional genes. The proteins of the subject invention can also be identified based on their immunoreactivity with certain antibodies.

[00067] The polynucleotides and proteins of the subject invention include portions, fragments, variants, and mutants of the full-length sequences as well as fusions and chimerics, so long as the encoded protein retains the characteristic biological activity of the proteins identified herein. As used herein, the terms "variants" or "variations" of genes refer to nucleotide sequences that encode the same proteins or which encode equivalent proteins having equivalent biological activity. As used herein, the term "equivalent proteins" refers to proteins having the same or essentially the same biological activity as the exemplified proteins.

[00068] It will be apparent to a person skilled in this art that genes within the scope of the subject invention can be identified and obtained through several means. The specific genes exemplified herein may be obtained from root-knot nematodes. Genes, or portions or variants thereof, may also be artificially synthesized by, for example, a gene synthesizer.

[00069] Variations of genes may be readily constructed using standard techniques such as site-directed mutagenesis and other methods of making point mutations and by DNA shuffling, for example. In addition, gene and protein fragments can be made using commercially available exonucleases, endonucleases, and proteases according to standard procedures. For example, enzymes such as *Bal31* can be used to systematically cut off nucleotides from the ends of genes. In addition, genes that encode fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these proteins. Of course, molecular techniques for cloning polynucleotides and producing gene constructs of interest are also well known in the art. *In vitro* evaluation techniques, such as MAXYGEN's "Molecular Breeding" can also be applied to practice the subject invention.

[00070] Other molecular techniques can also be applied using the teachings provided herein. For example, antibodies raised against proteins encoded by

polynucleotides disclosed herein can be used to identify and isolate proteins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the proteins that are conserved and most distinct from other proteins. These antibodies can then be used to specifically identify equivalent proteins by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to proteins encoded by polynucleotides disclosed herein, or to equivalent proteins, can readily be prepared using standard procedures known in the art. The genes that encode these proteins can be obtained from various organisms.

[00071] Because of the redundancy of the genetic code, a variety of different DNA sequences can encode the amino acid sequences encoded by the polynucleotide sequences disclosed herein. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding proteins having the same, or essentially the same, amino acid sequence. These variant DNA sequences are within the scope of the subject invention. As used herein, reference to "essentially the same" sequence refers to sequences that have amino acid substitutions, deletions, additions, or insertions that do not materially affect biological activity. Fragments retaining the characteristic biological activity are also included in this definition.

[00072] A further method for identifying genes and polynucleotides (and the proteins encoded thereby) of the subject invention is through the use of oligonucleotide probes. Probes provide a rapid method for identifying genes of the subject invention. The nucleotide segments that are used as probes according to the invention can be synthesized using a DNA synthesizer and standard procedures.

[00073] The subject invention comprises variant or equivalent proteins (and nucleotide sequences coding for equivalent proteins or for inhibitors of the genes encoding such proteins) having the same or similar biological activity of inhibitors or proteins encoded by the exemplified polynucleotides. Equivalent proteins will have amino acid similarity with an exemplified protein (or peptide). The amino acid and/or nucleotide identity will typically be greater than 60%. Preferably, the identity will be greater than 75%. More preferably, the identity will be greater than 80%, and even more preferably greater than 90%. Most preferably, the identity will be greater than 95%. RNAi molecules will also have corresponding identities in these preferred ranges. These

identities are as determined using standard alignment techniques for determining amino acid and/or nucleotide identity. The identity/similarity will be highest in critical regions of the protein or gene including those regions that account for biological activity or that are involved in the determination of three-dimensional configuration that is ultimately responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Below is a list of examples of amino acids belonging to various classes

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

[00074] In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not detract from the ability to manage nematode-caused diseases.

[00075] An "isolated" or "substantially pure" nucleic acid molecule or polynucleotide is a polynucleotide that is substantially separated from other polynucleotide sequences which naturally accompany a nucleic acid molecule. The term embraces a polynucleotide sequence which was removed from its naturally occurring environment by the hand of man. This includes recombinant or cloned DNA isolates,

chemically synthesized analogues and analogues biologically synthesized by heterologous systems. An "isolated" or "purified" protein, likewise, is a protein removed from its naturally occurring environment.

[00076] Recombinant hosts. The genes, antisense, and RNAi polynucleotides within the scope of the present invention can be introduced into a wide variety of microbial or plant hosts. Plant cells can be transformed (made recombinant) in this manner. Microbes, for example, can also be used in the application of RNAi molecules of the subject invention in view of the fact that microbes are a food source for nematodes

[00077] There are many methods for introducing a heterologous gene or polynucleotide into a host cell or cells under conditions that allow for stable maintenance and expression of the gene or polynucleotide. These methods are well known to those skilled in the art. Synthetic genes, such as, for example, those genes modified to enhance expression in a heterologous host (such as by preferred codon usage or by the use of adjoining, downstream, or upstream enhancers) that are functionally equivalent to the genes (and which encode equivalent proteins) can also be used to transform hosts. Methods for the production of synthetic genes are known in the art.

[00078] Where the gene or polynucleotide of interest is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, certain host microbes are preferred. Certain microorganism hosts are known to occupy the phytosphere, phylloplane, phyllosphere, rhizosphere, and/or rhizoplane of one or more crops of interest. These microorganisms can be selected so as to be capable of successfully competing in the particular environment (crop and other habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing a polypeptide of interest, and, desirably, provide for improved protection of the protein/peptide from environmental degradation and inactivation.

[00079] A large number of microorganisms is known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, *e.g.*, genera *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilus*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*,

*Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, *e.g.*, genera *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are the pigmented microorganisms.

[00080] Methods of the subject invention also include the transformation of plants or plant tissue with genes which encode the RNAi molecules of the present invention. In one embodiment, the transformed plant or plant tissue expresses antisense RNA and/or RNAi. Transformation of cells can be made by those skilled in the art using standard techniques. Materials necessary for these transformations are disclosed herein or are otherwise readily available to the skilled artisan.

[00081] Additional methods and formulations for control of pests. Control of nematode pests using the RNAi molecules of the instant invention can be accomplished by a variety of additional methods that would be apparent to those skilled in the art having the benefit of the subject disclosure. A "cocktail" of two or more RNAi molecules can be used to disrupt one or more of the genes identified herein. The "cocktail" of RNAi molecules may be specific to segments of a single gene or the entire gene. A "multigene cocktail" of RNAi molecules specific to two or more genes (or segments thereof) is also encompassed by the instant invention. In another embodiment of the instant invention, the disclosed RNAi molecules, cocktails, and/or multigene cocktails thereof, may be used in conjunction with other known nematode control agents and methodologies. Such cocktails can be used to combat the development of resistance by nematodes to a certain inhibitor or inhibitors.

[00082] Compositions of the subject invention which comprise RNAi molecules and carriers can be applied, themselves, directly or indirectly, to locations frequented by, or expected to be frequented by, nematodes. Microbial hosts which were transformed with polynucleotides that encode RNAi molecules, express said RNAi molecules, and which colonize roots (*e.g.*, *Pseudomonas*, *Bacillus*, and other genera) can be applied to the sites of the pest, where they will proliferate and be ingested. The result is control of the pest. Thus, methods of the subject invention include, for example, the application of recombinant microbes to the pests (or their locations). The recombinant microbes may also be transformed with more than one RNAi molecule thereby delivering a "cocktail" of RNAi molecules to the nematode pests. A carrier may be any substance suitable for



delivering the RNAi molecules to the nematode. Acceptable carriers are well known in the art and also are commercially available. For example, such acceptable carriers are described in E.W. Martin's *Remington's Pharmaceutical Science*, Mack Publishing Company, Easton, PA.

[00083] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

[00084] Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

#### Example 1— Production of Hairy Roots for RNAi Testing

[00085] A hairy root assay system was developed for testing the anti-nematode activity of RNAi molecules.

[00086] *Agrobacterium rhizogenes*: Several *Agrobacterium rhizogenes* strains produce hairy roots on a variety of plant species. *A. rhizogenes* strains, A4, 15834, 8196 and LBA4404 demonstrate hairy root development on tomato and sugar beet, with A4 being the most efficient. The *A. rhizogenes* strain K599 demonstrated very efficient formation on transgenic soybean hairy roots and was also effective on sugar beet and *Arabidopsis*. However, stain K599 failed to produce hairy roots on tomato tissues possibly due to hyper-virulence.

[00087] Hairy root production: Transgenic hairy roots were identified by stable GUS expression in tomato, sugar beet, soybean and *Arabidopsis*. The construct pAKK1401 (pNOS / NPT-II / tNOS // pSU / GUS / tNOS) was used to produce hairy roots when transformed into *A. rhizogenes* strains A4 or K599. Transgenic roots were identified by GUS expression.

#### Example 2 – Protocol for Electro-competent *Agrobacterium* and Electroporation

[00088] Electro-competent *Agrobacterium* Protocol:

- [00089] 1. Grow *Agrobacterium* overnight in 5 mls LB + antibiotics at 30°C on shaker (for *Agrobacterium rhizogenes* strain K599 no antibiotics are needed).
- [00090] 2. Use the 5 mls of overnight culture to inoculate 500 mls LB + antibiotics at 30°C on shaker. Grow overnight.
- [00091] 3. Add liquid culture in eight 50 ml polypropylene orange cap tubes.
- [00092] 4. Centrifuge 10 min., 4000 rpm, 4°C.
- [00093] 5. Resuspend cells in each tube with 20 mls 10% glycerol (on ice)
- [00094] 6. Centrifuge 10 min., 4000 rpm, 4°C.
- [00095] 7. Resuspend cells in each tube with 10 mls 10% glycerol (on ice).
- [00096] 8. Centrifuge 10 min., 4000 rpm, 4°C.
- [00097] 9. Resuspend cells in each tube with 2 mls 10% glycerol (on ice).
- [00098] 10. Aliquot 50 µl into cold Eppendorf tube and place onto dry ice.
- [00099] 11. Store electro-competent cells at -80°C. These cells can be used for up to two years.

[000100] Electroporations:

- [000101] 1. Add 1 µl to 5 µl of DNA (resuspended in H<sub>2</sub>O and not TE or other buffer) to 50 µl of *Agrobacterium* electrocompetent cells and mix.
- [000102] 2. Transfer 20 µl of DNA/*Agrobacterium* mix to cuvette.
- [000103] 3. Electroporate:  
25µF, 400 Ω resistance, 2.5 volts (0.2cm cuvette) or 1.8 volts (0.1cm cuvette for BioRad electroporator. 330 µF, 4000 kΩ, low w, fast charge rate for BRL Electroporator.
- [000104] 4. Add 1ml of LB and transfer to Eppendorf tube.
- [000105] 5. Shake at 30°C for 2 hours.
- [000106] 6. Centrifuge down cells (2 min. 14 krpm).
- [000107] 7. Plate all onto LB + antibiotics (most *Agrobacterium* strains are naturally streptomycin resistant).

Example 3 – Protocol for Production of Transgenic Hairy Roots on Soybean

[000108] Seed Sterilization. Rinse the soybean seed with 70% ETOH for 2-5 min. Remove and add 20% Clorox and shake for 20-25 min. Rinse 3X with sterile water. Plate the seed, 5 seed per plate, onto  $\frac{1}{2}$  MSB5 + 2% sucrose + 0.2% gel (referred to as  $\frac{1}{2}$  MSB5). Place seed into chamber at 25°C, 16/8 photoperiod for 5-7 day (depending on genotype) germination period. After 1 week seedlings can be placed into cold room for longer storage if necessary (not to exceed 2 weeks).

[000109] Agrobacterium Preparation. For *Agrobacterium rhizogenes* strain K599, take a small sample from frozen glycerol into 25-50 ml of NZYM media with 50 mg/L kanamycin in a 125-250 ml Erlenmyer flask. Place onto shaker at 28-30 °C for 16 - 20 hours. Pour sample into centrifuge tube and centrifuge the bacterium at 4000 rpm for 10 min. Pour off supernatant and re-suspend the pellet with an equal volume of liquid  $\frac{1}{2}$  MSB5 + 200  $\mu$ M acetosyringone. Use pipette to re-suspend the pellet and homogenize the sample (remove all clumps). To determine O.D., prepare a 1:10 dilution by putting 900  $\mu$ l  $\frac{1}{2}$  MSB5 into cuvette and add 100  $\mu$ l of bacterial sample. Determine the O.D.<sub>660</sub> and calculate the volume needed to adjust (dilute) OD to approximately 0.2 for inoculation. Check final O.D.

[000110] Explant Preparation and inoculation. Place a sterile filter paper onto plates of  $\frac{1}{2}$  MSB5. Cut soybean cotyledons just above the shoot apex and place onto plate. Lightly scar the cotyledon's abaxial surface (flat side, upper surface that reaches toward sun) with a scalpel blade. Cut each cotyledon transversely into 2-3 pieces (no smaller than 1 cm). Add approximately 10 ml of prepared bacterial solution to each plate and allow cotyledons to incubate for 1 hr. Remove the bacteria using a vacuum aspirator fitted with sterile pipette tip, ensure that there is no standing liquid. Orient all explants with abaxial surface up and wrap plates for a 3 day co-culture, 25°C in light (16/8 photoperiod).

[000111] Hairy root selection and maintenance. After 3 day co-culture, wash explants with liquid  $\frac{1}{2}$  MSB5 + 500 mg/L carbenicillin. Transfer the explants abaxial side up to selection media,  $\frac{1}{2}$  MSB5 supplemented with 500 mg/L carbenicillin and 200 mg/L kanamycin. Roots should develop in approximately 2-3 weeks. The roots will form primarily from the cut vascular bundles with other roots developing from the small cuts on cotyledon surface. Remove roots (>1cm in length) and place onto replica media with

transfers to fresh media every 2 weeks to prevent *Agrobacterium* overgrowth. After 6-8 weeks on selection the roots can be moved to media without kanamycin, however carbenicillin must remain in media for several months for continued suppression of *Agrobacterium*. At this stage roots can be used for testing RNAi for nematode control. Sterilized nematodes can be added and observed for RNAi affects.

#### Example 4 – Testing of RNAi for Plant Parasitic Nematode Control.

[000112] Various types of nematodes can be used in appropriate bioassays. For example, *Caenorhabditis elegans*, a bacterial feeding nematode, and plant parasitic nematodes can be used for bioassay purposes. Examples of plant parasitic nematodes include a migratory endo-parasite, *Pratylenchus scribneri* (lesion), and two sedentary endo-parasites, *Meloidogyne javanica* (root-knot) and *Heterodera schachtii* (cyst).

[000113] *C. elegans*: RNAi vectors can be tested through expression of the RNAi in *E. coli*. *C. elegans* are fed *E. coli* and assayed for their growth by measuring growth of nematodes, production of eggs and viability of offspring. Another approach is to inject dsRNA directly into living nematodes. Finally, soaking nematodes in a solution of *in vitro*-prepared RNAi can quickly establish efficacy of treatment.

[000114] *P. scribneri*: The *P. scribneri in vitro* feeding assay uses a corn root exudate (CRE) as a feeding stimulus and both the red dye Amaranth or potassium, arsenate as feeding indicators. Feeding is confirmed after seven days by the presence of red stained intestinal cells in live worms exposed to the Amaranth or death of worms exposed to arsenate. This bioassay is used to test soluble toxins or RNAi. *P. scribneri* has also been cultured on wild type roots of corn, rice and *Arabidopsis*, and on A. rhizogenes-induced hairy roots of sugar beet and tomato. *P. scribneri* is very valuable in evaluating transgenic hairy roots because of the non-specific feeding of these worms.

[000115] *M. javanica*: Nematode eggs are sterilized using bleach and are used to inoculate hairy roots expressing RNAi. Nematodes are assessed for their growth by measuring knots, egg masses or production of viable eggs. An alternative approach is to microinject dsRNA directly into root feeding sites or into living female nematodes.

[000116] *H. schachtii*: Cultures of this nematode were maintained on sugar beets. Nematodes eggs are sterilized using bleach and used to inoculate hairy roots

expressing RNAi. Nematodes can be assessed for their growth by measuring knots, egg masses or production of viable eggs.

#### Example 5 — Plant Expression Vectors for RNAi

[000117] Modular Binary Construct System (MBCS): An important aspect of the subject disclosure is the Modular Binary Construct System. The MBCS eases the burden of construct development by creating modular pieces of DNA that can be easily added, removed, or replaced with the use of low frequency cutting restriction enzymes (8-base cutters). These constructs are useful for delivery of a variety of genes to plant cells and is not limited to the delivery of RNAi genes. To develop this system, a series of six, 8-base cutter restriction enzyme sites was placed between the left and right Ti borders of a previously created kan<sup>R</sup>/tet<sup>R</sup> binary plasmid (Figure 1). The production of both kan<sup>R</sup> and tet<sup>R</sup> MCBS aids the testing of constructs using different strains of *Agrobacterium rhizogenes* in different plant species. In addition to the MBCS, a series of shuttle vectors were created that aid in the cloning of useful DNA fragments by containing the multi-cloning site (MCS) of a modified Bluescript plasmid flanked by 8-base restriction sites (Figure 2). With six 8-base cutter sites, each site is, preferably, reserved for a particular function (Figures 3 and 4). Because of the close proximity of the *Pme* I and *Sgf* I sites to the left and right border of the binary vector, these sites are, preferably, reserved for gene tagging and enhancer trap experiments. The *Not* I site is, preferably, reserved for plant selectable markers (Figure 5). The *Pac* I site is reserved, preferably, for Plant Scorable Markers (Figure 6). The *Asc* I site is, preferably, reserved for RNAi experiments (Figures 7 and 8), while the *Sbf* I site is, preferably, reserved for anti-nematode proteins. The restriction sites that are denoted in the Figures are, preferably, reserved for the denoted insertions; however, the MCBS binary and shuttle vectors do not require the restriction sites to contain these suggested inserts.

[000118] Plant Selectable Markers for MBCS: To further develop the MBCS, a series of plant selectable markers were added to the MBCS (Figure 5). Plant selectable markers that were added to the MBCS include: pNOS/NPT-II/tNOS (kan<sup>R</sup>), pNOS/Bar/tNOS (basta<sup>R</sup> for dicots), pUBI/Intron-Bar/tNOS (basta<sup>R</sup> for monocots), and pUBI/Intron-PMI/tNOS (mannitol isomerase<sup>R</sup>).

[000119] Reporter Genes for MBCS: Four exemplary reporter genes are used in the MBCS are provided in Figure 6 and Appendix 2. GUS, a nuclear localized GUS, GEP, and the anthocyanin transcriptional activator *papIC* genes into the MBCS.

[000120] Promoters for MBCS: We cloned several useful constitutive and nematode-inducible promoters (Figures 6, 7 and Appendix 2). Constitutive promoters include the SuperUbiquitin promoter from pine (pSU) and two promoter regions from the Strawberry Banding Vein virus (pSBV<sub>1</sub> and pSBV<sub>2</sub>). Seven nematode-inducible promoters from *Arabidopsis* were also been cloned.

[000121] The following Scorable marker clones have been constructed and placed in the MBCS, NPT-II binary vector (pNOS/NPT-II/tNOS):

Intron/GUS/tNos	Intron/NLS-GUS/tNOS	Intron/GFP/tNOS
pSU/Intron/GUS/tNOS	pSU/Intron/NLS-GUS/tNOS	pSU/Intron/GFP/tNOS
pSBV <sub>1</sub> /Intron/GUS/tNOS	pSBV <sub>1</sub> /Intron/NLS-GUS/tNOS	pSBV <sub>1</sub> /Intron/GFP/tNOS
pSBV <sub>2</sub> /Intron/GUS/tNOS	pSBV <sub>2</sub> /Intron/NLS-GUS/tNOS	pSBV <sub>2</sub> /Intron/GFP/tNOS
pKT/Intron/GFP/tNOS		
pKA/Intron/GFP/tNOS		

#### Example 6 — Control of Plant parasitic nematodes using RNAi *in planta*

[000122] Production of RNAi Vector. The RNAi shuttle vector to be used is adapted from the Modular Binary Construct System (MBCS - See Example 5). RNAi shuttle vectors preferably comprise a promoter, intron, antisense RNAi, stuffer fragment, sense RNAi, and terminator (See Figures 7 and 8 and Appendix 2 for more details). The plant promoter can be constitutive, tissue-specific or nematode-inducible. The intron is necessary to eliminate expression in *Agrobacterium*.

[000123] The anti-sense and sense RNAi molecules comprise nematode-specific sequences and are disclosed herein. These genes are associated with pathogenesis, growth, or other cellular function in nematodes. An exemplary group of RNAi sequences for use in plant/nematode control may be based upon:

[000124] 1. Genes specific for nematode esophageal gland cells.

[000125] 2. Genes specific for plant parasitic nematodes but not other free living nematodes.

- [000126] 3. Genes common to all plant parasitic nematodes.
- [000127] 4. Genes common to all nematodes (nematode-specific).
- [000128] 5. Genes specific for important tissues or cell types.
- [000129] 6. Genes from large gene families.
- [000130] 7. Genes involved in nematode signal transduction or other cellular pathways.

[000131] Appropriate RNAi constructs allow for the formation of dsRNA molecules (the sense and antisense strands join to form the dsRNA). The terminator sequence adds a poly-A tail for transcriptional termination. The RNAi shuttle vector can then be subcloned into the MBCS and transformed into *Agrobacterium rhizogenes*.

[000132] Plant Transformation with RNAi Vectors. An exemplary transformation system for generating hairy roots using *Agrobacterium rhizogenes* is provided below. The RNAi vector once introduced into the MBCS can subsequently (as a binary vector) be transformed in *A. rhizogenes* using, for example, the electroporation protocol of Example 2. Once the *A. rhizogenes* is confirmed to contain the plasmid, it is then used in generating hairy roots (See Example 3). Using this protocol transgenic hairy roots expressing RNAi are isolated, cultured and tested.

[000133] Testing of RNAi Vector for Nematode or Plant Pathogen Resistance. RNAi expressing hairy roots can be inoculated with sterilized nematodes. Infested hairy roots can be observed and the effect on nematodes determined. An alternative approach involves the microinjection of RNAi directly into root feeding sites (giant-cells for root-knot nematode, and syncytia for cyst nematodes) or into living female nematodes.

#### Example 7 – Insertion of Genes Into Plants

[000134] One aspect of the subject invention is the transformation of plants with genes encoding proteins of the present invention. Transformation of plants as described herein can be used to improve the resistance of these plants to attack by the target pest.

[000135] Genes, polynucleotides, and/or RNAi molecules as disclosed or suggested herein can be inserted into plant cells using a variety of techniques which are

well known in the art. For example, a large number of cloning vectors, for example, pBR322, pUC series, M13mp series, pACYC184, pMON, *etc.*, are available for preparation for the insertion of foreign genes into higher plants via injection, biolistics (microparticle bombardment), *Agrobacterium tumefaciens*, or *Agrobacterium rhizogenes*-mediated transformation, or electroporation as well as other possible methods. Once the inserted DNA has been integrated into the genome, the genetically modified-cell(s) can be screened via a vector carried-selectable marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G418, bleomycin, hygromycin, chloramphenicol, or bialophos, *inter alia*. The transformed cell will be regenerated into a morphologically normal plant. The transgene(s) in the transgenic plant is relatively stable and can be inherited by progeny plants.

[000136] If a transformation event involves a germ line cell, then the inserted DNA an corresponding phenotypic trait(s) will be transmitted to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

[000137] It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.



We claim:

1. An RNAi molecule, optionally comprising a linker, wherein at least one strand of said RNAi is encoded by a DNA sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 139.

2. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 1.

3. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 2.

4. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 3.

5. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 4.

6. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 5.

7. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 6.

8. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 7.

9. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 8.

10. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 9.

10. 11. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
10.
11. 12. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
11.
12. 13. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
12.
13. 14. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
13.
14. 15. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
14.
15. 16. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
15.
16. 17. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
16.
17. 18. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
17.
18. 19. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
18.
19. 20. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
19.
20. 21. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
20.

21. 22. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 21.
22. 23. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 22.
23. 24. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 23.
24. 25. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 24.
25. 26. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 25.
26. 27. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 26.
27. 28. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 27.
28. 29. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 28.
29. 30. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 29.
30. 31. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 30.
31. 32. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 31.

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32. 33. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

33. 34. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

34. 35. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

35. 36. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

36. 37. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

37. 38. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

38. 39. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

39. 40. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

40. 41. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

41. 42. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

42. 43. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

43. 44. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 43.
44. 45. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 44.
45. 46. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 45.
46. 47. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 46.
47. 48. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 47.
48. 49. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 48.
49. 50. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 49.
50. 51. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 50.
51. 52. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 51.
52. 53. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 52.
53. 54. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 53.

54. 55. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
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55. 56. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
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56. 57. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
56.
57. 58. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
57.
58. 59. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
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59. 60. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
59.
60. 61. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
60.
61. 62. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
61.
62. 63. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
62.
63. 64. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
63.
64. 65. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
64.

65. 66. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 65.
66. 67. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 66.
67. 68. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 67.
68. 69. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 68.
69. 70. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 69.
70. 71. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 70.
71. 72. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 71.
72. 73. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 72.
73. 74. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 73.
74. 75. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 74.
75. 76. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 75.

76. 77. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
77. 78. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
78. 79. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
79. 80. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
80. 81. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
81. 82. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
82. 83. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
83. 84. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
84. 85. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
85. 86. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
86. 87. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:



87. 88. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
87.
88. 89. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
88.
89. 90. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
89.
90. 91. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
90.
91. 92. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
91.
92. 93. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
92.
93. 94. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
93.
94. 95. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
94.
95. 96. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
95.
96. 97. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
96.
97. 98. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
97.

99. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 98.

100. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 99.

101. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 100.

102. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 101.

103. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 102.

104. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 103.

105. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 104.

106. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 105.

107. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 106.

108. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 107.

109. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 108.

110. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 109.

111. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 110.

112. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 111.

113. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 112.

114. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 113.

115. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 114.

116. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 115.

117. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 116.

118. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 117.

119. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 118.

120. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 119.

121. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 120.

122. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 121.

123. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 122.

124. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 123.

125. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 124.

126. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 125.

127. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 126.

128. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 127.

129. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 128.

130. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 129.

131. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 130.

132. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 131.

133. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 132.

134. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 133.

135. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 134.

136. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 135.

137. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 136.

138. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 137.

139. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 138.

140. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 139.

141. A transgenic plant or transgenic plant tissue comprising an RNAi molecule according to any of the preceding claims.

142. A method of disrupting cellular processes in a nematode comprising the steps of:
- (a) providing a composition comprising a compound according to any of the preceding claims; and
  - (b) contacting a nematode with said composition.

143. An isolated promoter comprising the following nucleotide sequence:

aacagcccaagataaacagaaaagtcaaagggtgttcgaaa  
gaccacttgtgactaaggatcatttcatccataattatctggtagca  
cagactcatgataaetgcgaggaacacaagttctttacagtcgattc  
aaagacactttctctttacggtttcattgaaggagccgaccagaat  
atgtcagagaagcttttctactgtgggttaatttcattaatctatcca  
ggtgaaaacctcaaggagatctctcttctccaaaagacctctacag  
ggcaatcaaaaactacagaaccagagtttgtagtgcacagagtagac  
caatctacctgagaatcacgagtaccttcctagagtgggaaaatgat  
gacatccttattccataaccactggattgaggtaggactatccaatgg  
aaaaattccatgggacaagtcataataagaagaccgcaacagtcgagt  
atcttccagagataactgcactcagacctaaaaggataaaaagcagta  
tataatcagtgtactaagatcttcgcagattcaaagaagaagcttaa  
ctatgctgatgacaagataattctaataagcaattattcagaattaa  
tcaaggagaaagaattaataactctttcagaatatgaagcccgcttt  
acaagtggccagctagctatcactgaaaagacagcaagacaatggtg  
tctcgatgcaccagaaccacatctttgcagcagatgtgaagcagcca  
gagtgggtccacaagacgcactcagaaaaggcatcttctaccgacaca  
gaaaaagacaaccacagctcatcatccaacatgtagactgtcgttat  
gcgtcggctgaagataagactgaccccaggccagcactaaagaagaa  
ataatgcaagtggctcctagctccacttttagctttaataattatgttt  
cattattattctctgcttttgctctctatataaagagcttgatattt  
catttgaaggcagaggcgaacacacacacagaacctccctgcttaca  
aaccatgtattgtagctaaacctcttaggag.

144. An isolated promoter comprising the following nucleotide sequence:

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tggtggggacaatggatccggtctgcgtagcaacaaggctg
aaaaagattaaacagaaacctgtgatcattagcgttggaccaccacc
aaaacctcctgagccaccaaaagcctccagagcctgaaaaaccaaagc
ctccaccagcacctgaaccaccaaaagcatgtatgcaagccaccttac
tgcaacagttgtgatgttgtgtctgttactacctatgaaagtggaag
cggctgcaccattctttgagtcataatcgcgtagcatagccttcat
gttaagtcctgtatttagccaataactaattcatcatgttctcatgct
tttttggtttatttctttttctcaaatatgaatctctgttgtttgtcc
ctccctgtttataatttagtcgcttctttgacacaagaagtctcatg
agttcatgctaaagaaaaataaaagttcaaattaaaacaccaaagtgtt
tgattaatttccataaacctgtgaagcagaaagttagtcattgttgac
ctgaacagagccttaggaagtccttgaaggacatatcttcaagtgtta
ttgggtcgtagcactcttaggccatttaacttcattgagccatttaa
attatgcaaaacaagaaatgagacatatggaaacattagggttctta
caggaaaaaataggaaaaagcagggacaactaaacaaaaattcagaa
acaagaggcaagtggacgaccacggcgtaagatcaacatgtggtgat
gtgcatgagaccaagaccattttttctcgttcttcaacgcacacttg
gtcttttcttatgtttgttgcatttctttatttaggcagaccctctct
cttttttaatataggatagtaaaaaatatatgattttattttgttgaaa
cattttgaggttaaaacctaaacttatagtaagcattttagtagagttaa
tttctatagcagatctatcaacatgaccttaacaaaaaaatatt
gatgaaactactttaagtagtaaaacctaaagcaattaaaatttctt
ttaaattagtagtttgtgtaaattaatgacatgattgcgtcgaaag
aaatcaaaacagttatatcgtagaacttaggagaatgttttatatcgt
gtttcaacacatgattgctagcatatgtgtagggtgcgtagacgtta
cataacaatcatcactcgtaaatatcaaagtgggttctgagagaaac
aaagggttatgattttcccaactgcactagttgtgtattgtttcttt
cacacgtatgcttctgagttctgcccagggtggaattaaagcagag
ttgggagagatcataatttatttaggggttcgttatgctcaagtcattga
cgtaaaatgaaaatttgtttttattctttcaccaacacaaagaatag
ctagttatctctttttttatatataacaattcatgaagttgatcagc
tttatacacatcatccaatcgaattgctaattctagagatggaaatat
caggatagagccaataagatatcaaattcaatggaccattttctcc
atgtgctaattcatacaatctgtttttgtctgctttattttgatgatg
atgctgagcgttttttaagtgtgaactaagatctagctaaccacaaaca
aagatgggtctcttctgtctttgtcgtataagagcaagagagtggttt
gattcaatttttaaaattctaaataaaaactccaaccgtgaatccagc
catgaaaactcttttttagaaaatccttttttataacaaataattcctc
tgcttcttcttcttcttctgtttatttcaccttttttggtttcttttag
ctcagaaaaagcccattctttttttctattcttgtttattttaatca
tactgtgcgtttctacaaagtttgttcctttcttcttcaactctctc
actcacagtcacagagatctgtttctttttcttttttttgctttcactc
ttctcttccagt.
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145. An isolated promoter comprising the following nucleotide sequence:

agcaaagcaagaacaccagagaagaagaaaagcactacaga  
gaaaaatgtgagcttaagcgcctctccaacaacacttctctgggagtc  
taaaggatgctgcaaaaagccttggtggtgagacttccgcataatttc  
caagcatgggtttatTTTTgttagcacacaaactatctgaccctcga  
cttggatTTTTcttctgcagtttgtccaactacattgaaacggatatg  
caggcaacatgggatcatgaggtggccatctcgtaagattaacaaag  
tgaacaggtcactaaggaaaatacagacgggtactggactcgggtccaa  
gggtgtagaaggaggactaaagtctgactcagcaactggcgaattcat  
tgcagttagaccttttattcaagaaattgatacccaaaagggctctgt  
cgtctcttgataatgatgcacatgcaagaagaagt caggaggatatg  
cctgacgatacttcattcaagctccaggaagctaaatctgtcgacaa  
tgccattaagttagaggaggatataccatgaatcaagcaagaccag  
gtaagaacttctctatccataaaccatagatggagcgttagaatct  
taatccatTTTTcagtttttgcaggatcattcatggaggttaatgcta  
gtggtcagccatgggcttggtggccaaagagtctggcttgaatggc  
agtgaaggaataaagagcgtttgcaacttaagctctgtggaaatttc  
agatggaatggatccaacaatccgatgcagtggcagtatgttgaaac  
ctaaccaatccatgtcatgcagcatatcagattcatcaaattggctca  
ggcgcagttctgcgtggaagctcatctacttccatggaagattggaa  
ccaaatgagaaccacacacagtaatagcagcgagagtggatcaacaa  
cgctgatcgtaaaaggccagttatagagaagacactgtacgtttcaag  
ttcgagccatcagttgggtgtcctcagctctacaaagaagttggaaa  
acgttttaaaactgcaggacgggtcgtttcagctgaagtacttggatg  
atgaagaagaatgggtgatgctgggttacagattctgatctccaagaa  
tgtttgagatatattacatgggtatgggaaaacactcgggtgaagtttct  
cgttcgtgatttgtctgccccctctaggtagttctgggtggcagtaatg  
gttatcttggaacaggcttatgacgtcgtaagacatagacacacaca  
gttatgtattcccagtgaaagaatgttggtttatttctctagatatta  
gtatgcttataaataggcatgaaggagaaagacaattttgggtatagt  
ggagttcagcagaaaatgtatatgtttttcgttttatatgaatcag  
agaataaaaagttggatgttatatctacgttgctaattgttgtaacctgc  
tcacccatctttcatataagaaaagagaaacacttttagttatccctg  
tgatgcagaatcgtattctttgttatctctccattcctgtggaaacc  
aacaagtcaactaaatttcggtttaattgggttggtttttaagtcaa  
cgaggacttgatttttagttgggcttgggcctataattgtgttcatca  
ttgggttttttcccccttatcagtttaacgtccatatccatatcttt  
ttcttttttaacggcaagttcatatccatatcttatgatgtgcct  
aaaagagggagaagatgcaagacagaattttcatatttgaaaggggt  
tcgatatcgatatgtgggaaacgaatcaaggtcaaaaaactcagtcata  
atagttgaaatttaaaaattttattaattcaatccgattgggttcgt  
tttggttatgggttcggttctatatcatcaaaccaatcggtttgggtcct  
aaagataattataaatattcaccaacaccagtggttaaacacatatca  
acaaacctaaagttagataaacaagaga.



146. An isolated promoter comprising the following nucleotide sequence:

```
aat tgg cact ctt ctt ctt gct ggg ttc caa aag aa ac ga at
ca at at gt gca aca aga ag ag ct cc aga ag cag tca tct tct aa at
ct ta at cta aca ac ag ct ca aga a gaa a a a a a t tcc at ag ct ag a ga
ga ac aca a ag tca ca ag ac ga cgt cgt ag ag gc aca a ag tca a ac ct
ga at gg ct ta ag cc ga act ga gt ggt ttt gact ag acc at cat caga
aa ag tct cca ag ac gg tag tcc gat gtt ag at cgt cca ag ta at ttt
tgg ttt tgt tgg tct cag ttt ttc ag ct gcc at ttt gat ttt cag ttt
ggg ctt ttc ctt at ctt cta a ag gcc a at ttt catt ttag gtt tag ttt
at ttt gat catt at ctt tact a ta a ag gct tcc gct ttc gaga a at ttt
agg gtt tct tct gtt cgt cgt cact cag gtt tgt gc ct ca ac ga c
tg ctt cact tct ag ct tga tt tct tct tct cgt ttt at at gt ata ct g
ta catt ag att at tct tgt ttt cct g ag ct tct gct at ag att ttt gat
tct ttt ttt tgt tgt ctt tgt ttt cgt ttt cc ag gat cag at ctt ag ct
aa at tga ga ca ag ct caa a at ga ggt act tga cgc at ctt ctt ac att
cact gtt ta at tag a ga a ca at ac gt ctt ctt ga at cgt gat tca ga ga
cgt at tgt tct tct gtt cat at gca at a ag ttt a at tag a ga a ca at a
cgt ctt ctt ga at cgt gat tgt ttt ttt gg at gtt gc gtt at tga tag ct t
tat gat gtt a at ag tct ag gatt ga cac ga ag ttt gtt ctt gc ag ttt t
gc at aa at gct ctt tact a ag gct cta a at ttt gg at ga ca a at cta
aat ctt gc ct ca ta a a a at ttag gtt gta tta ag a ta ag att at ttt g
tat gg tag tgt cta at at gt ggg ttt gtt cat gtt ga ggt tgt ca at g
ttgt gta ttt ttt gtt ttt gtt tag tta at ttt gct ta act ctt gtt tct ttt g
tgg gtt a at a ca gta ag ct tca g ag tga ggc cgt tct gta ag cc at c
act act at ca cag gga a at cc gagg caa a ga a ac gta act ttt gtt cga
gact at tga gct cc ag at cgt ctt ga aga act at ga cc ct caa a ag g
aca ag cgt ttt cag tgg at ctt gtt ca ag tt acc a ca ta tcccc cgt cct
aaa at ga ag at ctt gca t gct cgg ag at gcc ag cat gtt ga ag ag gt
ga ta ta tct ttt cat gg aa at tga tca ttt ttt gtt gct ctt gtt tct ttt gtt
ata at ggt ttt gtt gct catt tct catt ttt ggt ggt ctt at tag ttt catt
tg at gtt gta ta at gt ctt ctt ga at gta g at gca t gat gtt ttt cgg aa
ttt ggt catt gtt ttt tag gct tca ttt ctt gca ta at ta a at att
tg ctt at ttt cat ctt gta tct ttt cgt ag gct gaga ag at ggg gtt g
gaa a ca tgg at gtt g ag tct cta a a a a ag ct ta a ca aga a ca ag aa
act cgt ca aga ag ct tgc aa a ga a at acc at gct ttt ctt ggc ct ctt g
agt ctt gtt catt a ag cag at tct cgt ctt ctt ggt cct ggt ctt aac
a ag gc ag gca ag ttt ctt gg ct a ca gct a at ttt cc att gtt ctt ctt t
ac at cc gtt ttt gat ttt gg at ag gtt ttt ag tag tct at ttt ctt ttt gtt
ca at gtt ctt ttt gata ca at gcc a at ctt ttt at cct gtt ga gatt at g
ctt ctt ttt gat gat tct ta ag ta ac att ctt ttt gct ttt act ttt a ca ca
gg aa a at tcc ca act ctt gtt ga gcc acc ag ga at cct tgg ag tca a a
ggt ga at ga a ca a ag gca ac ag tga ag ttt cc ag ct ga aga ag gtt c
tgt gca tgg g ag ttt gca gtt ggt a ac ctt t.
```

147. An isolated promoter comprising the following nucleotide sequence:

ttggcaaactgagatataagaggggaaggtgattttcatgcaa  
atTTTTTTTTTTTTTTTTgaatgaatgcaaaatttattcaaaaa  
aaaaaacctgggctacatcaagtacttcatttctgagtttttgaaa  
aatctaaagacaacaaaagactttacaatttaataaaaaaataataa  
aaatactttatcactctcaacgaaattgttgatttaataacgtatct  
cttggtaaaaacagcgttttatttgacgaaattgttataaatgaataa  
aatgataatagaaaactagtgtggtacgtaaaaatacctctcatttggc  
aaaataacggttatgtatcatgagatttgcatacgcacagcgtgctta  
aatagtgtgctttcaggagaaaaatataccaagttatttgctgaaa  
ttaccacgcaaactctgaggttcgaatggcaaaataaaaaaccaatgt  
catttcccttaatgtattaaggtcatttaataaaaattgtacactttt  
ttcacctgtaagcgttccaaagtgtagaatggataactagaagggtc  
aaaggtataatattaataagcgaactcactttttgcccaagtgattt  
cacttcttacatttgcttgatatagttacccaaaagtgtatatatat  
tcccttataacaattgttctattttctggattataaggggaataagaa  
aaaagaaaagagagagtatataataacttttataaagtgatgtta  
gatttctaatttgtaacgaaaagttcaaagtgaagaaaaaacgaaaa  
agtttttctgttttgttttatatctatagccaagaaagtttctcaga  
tttacaagaagttaactgagaaaaacaaaaaaaaaacttatgaagca  
tgaaagactaattaacgaggtgatttaattttgagacaaattaaacat  
cgaattaaaagtaacatttggagggtttatatgttatatatgtgaca  
tgataagtcaggattcatgactaatgtatatctggaatctaacatgga  
agaatagagaacgaagcagagccaaggtcaacttgccagacacgaat  
caacagatttgtgaatgagacaaatcaatgggtcataaacgggttggg  
tttaaaccgggcaagtcaccttgggtcaattccattcggtatttcctt  
catgcaagaccctctgatacaaccaaaagactcccattacaatatctt  
ttcgatcacgagctacttattttcaaagtgtttacctctttcggtgac  
tcttgtgttgtgtggttaaagcctagtcgagatgtgtcggtatatata  
ggcatacatatacaaatgacgacaaaataagtatatttatattgtttaa  
tttctatatattccatttctatatgcatgggtgggatttttgacaaaa  
ccctaattcaagaatagaatccaaaagatgggatcaaagaatataat  
ctaattgggctgaccacattttccgattttaattcgcatagttaatatt  
ctttccactactttatgccgcagaaatttgtaattaagtaagacaaa  
gaaatacagatatagatgggtcgtagaaaccagtagaggaatttcat  
tttctgtggataagtgggaatattaataagagaatgggtctttactctt  
tacagtgggaaatgggaatagtagccattataaatttcatcagattc  
tatatatgcatgtttgtataagctaaaataaatacgtttaagcattc  
ttcaaaaaaatttacaagttctagagactctcttaacgtcggcaatt  
tatattctactttacatgacactttcaggaaaaagaaaactataactca  
ctagcagatcattaaattttctttttctttttttgaatgaaccttag  
ttgtgggtttttatttttgttagctagaaacttcagtggtttttttcc  
gccaatggtagtgctttgatgatgggtccgg.

148. An isolated promoter comprising the following nucleotide sequence:

```
caatcaaggtaacgaaggaggatcagcgaaaggatgggcta
tatttggagttttttcctgcgtgtaagtaatgctttgtgatcttcca
tgcggacatataactgaagaataaactcaactcattgtgttctgggtg
tgtttcttctgatcagattcctcgttgcacatcgcacttttctgctgt
gggggctttatttataaaacaagagtagagcgtgtggtaatcttcat
atcttcttacaattccacttccattctcttaattattctctcacgtga
tatacacacactcaatcactgatgtactcgtatggatgcagcgtgga
actgatgcattggcggggatgtcacttctatcgggcttactagaaac
tgtaagtattacaagaaaactcaaaaggattccatttatgcaaaatc
taagagaaagctcactgtggtctttgggttacaatttatggatctctc
aagagacaaatgctatgtaagctaattgatttttgggtcttgataaaca
ggtagtggaagtggacaaagctactcaagaactgaagacatcaaca
atgcttttgccaatgaagtctcatgggaccgctcttccgcacatctct
actcaagcgacaacaacacagagaccaagtgaagaacatatgggtgc
gatctaattttgtcaagtgcctcacaagaggtagtgttcaagccat
ggtagggcacgcttgtgatctgcgatttctggattttgctttgtatg
tttattttctaccttctagaaagagggtcaaaaagttaatagcttcac
cgtgagaatgttgttttcaccagattcatgtgctatgatagaaaag
acaaagcaaacaagagttctttctttgcttaggttacaagaacaaga
gtatcgttataaagtcaacaaagattgaaacatatttttgtcaaggg
agtgggttagaatctcttctactctcttgcctttctcactaagacaa
aaaaaagacttggactttgtctaagggttttgtggatattattaacca
agtccttttgcaaaaagtaatatgttttttcgcattcctcttttag
aatttagtttaatctaggcctttatatgtggttattactttcttgaaaa
atgatctgtttattctattcacttgggttacctcgctttttatctt
acttctacaaaaggattatcagtgaaggttagtctcttactctcacc
ttccgaaaataaaaacaaaatatcgatacttctagatcaaaccaagt
tgattaaaacatccctattccctaagattctgatcttgagatatatt
atcatgttaagatctaaattgacaagaaaactgatttttcatctcta
gtaggaaaaataattactatttagtgatcatgattgtcgaccgtaaga
ggtaggttagttactctccatctttctttgaagaagtcagaaagtca
gaaattatatcaaattaacatcaatatgaacacatatatctgtat
ggttttatgtttagaaaattccaatatatttatattcctagggaaaa
agaagcttattcttcaaattattgttatgagtcgttaaaatatggat
aaaaatataaagtctaaatatataaaactcagttttgctttgctttta
cctctccaagctctccaagtcacaaatatttttagtttaattaaaccaa
aaaagggtttatttagtcaaacttagcatgcaatgctgggtaccaaacc
caagcatttagtctcttttaattctctttttctccaataagtttttac
aatttttaattgtttgcatttcccttgattatttatcttcatcccaa
tttagctaataccaactccgtttcttattcttccaagtccttttcta
taaatacgttcttcttccctcttatttcatatcactcaccacaaag
tcttctcatttctcat .
```

149. An isolated promoter comprising the following nucleotide sequence:

atgttgtgagtgaaggagaagaagagggaaacaaaggtatt  
tatttgtagcgagttttgtttgtgacgcggtttgtctgtgttcaa  
tggtgacgaaacgagtgagagagtgctgattattaaagaaaaccct  
aattaagtcagacccgcccgttataaaaatagtcaaaaagtaggaaa  
acgcgtgtgtgagtgagacagagacagcccattgtttgctttatggg  
cttataagcgcgagacgtgttaattgggctttttcctttatggccgaaa  
acaaaagaaacgtgcctgagagattcgaactctcgccgggcagagcc  
catgtacttagcaggcacacgccttaaccactcggccaaagcgactt  
gttgctatgagttagacaaaaatcattaaaattctctattatgatttc  
tcatagtgtgtgtgtatattgtggatctactaaaaattctttgttat  
tattacttttattttgtgaattagtttgatataggttaagtacaaagtt  
aactttattatttactcaaaaatttatcagattaactgattttatatt  
gtttccttttggtatatatagacgtactatagtttttagaaaaaccataa  
gattccttttatatttcatagagtgaagagatgagatgagatccttggc  
tgagagaagaaataagtttccacgaggaggactcttttttttttggtga  
agacgaggaggaggactcttggttgatccagtctttacgttagacat  
cgacccctacattttatttgcctttctctatcaacatggcaggtaaaa  
atcttcattcaaccgaaccaaccaaaagtctcttcccaataatattca  
agcaccatcctttgggaaactcacatactacagtctacactcttt  
cattttctttcaacgctcaacttaacaaatgatatagtctagttgtc  
aattatatgttttaattagtgttttcacatcaaattctggtttgata  
tttgatgactattttcggaacatctcaatgtcccgcacaaatacaatc  
gtctatcatatataatcccgtacgttgtattcttatagatagaataa  
tatggcgtgatctttataatataacatatagaatcgtgtagatttat  
tttattttatttttatatatcgcataaattgcaaaatacttatatat  
gtttgttatatatgatacccattttatagttacttaaaaaaagttaa  
gcgataatatatatatatcaactttttataacaaaaaagtataacac  
atggtaaaagaaaaataaaaatgaagacatgggtgtgacacgaaaatgg  
cactaaatatacatatataatagatagctacaatatcccatcataca  
cacttttttaattgactaataacataaacttacacacttttttaattga  
ctaattcataactttttatcattgtcaacatgcaaatcatatttcc  
gttgaactattattcttattttgtttttaaaagaagggtcttcctgggt  
aataaaaaatgatgtttccaaatgacgttagagcaaaaaaaaaaaaaaag  
gttgtctgggtctggtaaaatgaaaaagcaaagcgtcttgggtatagaa  
aagtaatatactgcctcctaatttcttctgctccttctaccgaagaatc  
tctccactcttgcctcttttcgaaaccctaaaccagaagcaccagat  
tttttcaactttttccagagagaacaatagaaaacccaacttgtgctc  
tctagggtttttctttattccttctcatctttggattttcttgggtca  
tcatttttggagcttaccaccagcgaaaaaattataacttccatcg  
attcctgggtctctctctctcgctctctctgcatgtgctaaatcgccg  
gactgatcctcactgtcacctctggt .

150. An isolated promoter comprising the following nucleotide sequence:

gattaggggtttgagttgtcactggaaagaggtttgattgt  
gagtgatgatggagagattatgaaggagtttgtgtgtatttatagag  
gagttaggggtttgaggtttgatgagaagtaggtttgaagaagtttt  
gttgttgcaacttatttagagttacttgttccacaaccacaagtaag  
attggtcacttctaagttctaactagaaacaacccatgacacatggag  
atttcagctaaccctagtttaattgtatatgtatttatattttatttaaa  
tattataaaaataaaaataaattttcacaaataaaaagaactacaaaaaa  
gtgagaaaaataatttgataaacaatttagaaaattagtatatcaa  
taataaaattttataatccgatgggttttgcccttttggtttggcctttg  
tttgaacttcgatgagtgactatgtatagcgaaaacaattccggtttg  
tttttggtttaatttttaaaaaatacaagcgacaatatctgatgagaa  
taggtgaaaagcaaataatatcagtttaattggaaatatctactttt  
ttacaactaatattttgtttgggtcaaccaacaatatagatttaattaa  
ttatgggttatgagctttttatttgttgcgacagtatatatatgttaa  
aatagtgatattgcatggcggaagggtccggaagcaacacatatctcc  
tttttaatttttttttaacaagaataacatgttaatttttttttgga  
aattaataaagaatacatatttctaatttttgcgtcagatagatgat  
taaagagtgtgtgttttttttaacaaacaaggaatacattatacata  
tttcataatttctctcgacattgtttgttttttaaaaaatagattaa  
agagtcctacgaagctaagtagctaacgaagacttgaaatgagaagaa  
gacgagaatcttttaatatattttttgttaagcgataatattttgaaaa  
ttaataaatatagattaaggaaataacaataacgcagatatcggttaa  
gtcatagaaaaaaagaaacaacacaaacttacataaacatgtttcct  
aatttglaatggagtaaaattccttcttttttttttttttttttgattt  
ggattccaattagtaaaagaactcaatgactataaataacctttaacc  
ctctcattatttcttactatcaattgatttaagctctcgttccctaaga  
aagcaatagacgaacaagaacccatcgaagaacacaaatctctcttt  
gaagttgtcgataatgttagtacaccgttacttcgtccaagactttt  
ttgccgttcggtttcttacaacaaaggatttggttaccattacttt  
tgtcgtaactcctttttacatgtacgtcaaaaagtgggttcctcgctc  
cggccttgaaagaaacgaccttcttaccacaaaaagcttatttttaaac  
cgtctaaaaccggaaaatctcaatctaaaccggatacgggttcatgag  
aaaccgattcaaacaccgagtgagaagtagaattttttgatgggttc  
cgtcacaatgtgtgctgctccttcgccaagacatgtaccgattccga  
tattttgtgggtgtaaagatgatcaaagagtccttcaaagctaagcacg  
acttgaatgagaagaagaagaccaattactcaattagattttgtttt  
gtggagcaattattgtctatttatctttgttttttagcaaataatctg  
tatccactaatcttcacagtacttgactaacaagaagtaaaagagttt  
tcttattttccaattgttttttaactctgatacttttttcataatttta  
caatgtttgatgaaaaaaaacattcaaacctaaaattttctttttttg  
gtatgaattcaaacctgaattacttttgacgaggaccgcgaggtata  
aatagggtgatctcccaacaacaaaaagggt.

151. A transgenic plant or transgenic plant tissue comprising an isolated promoter according to any of claims 143 through 150.

54  
**APPENDIX 1**

SEQ ID NO:	INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
1, 2, 3	2293133	glyceraldehyde-3-phosphate-dehydrogenase
4, 5, 6, 7	7143495	Histone H4
8 & 9	7143515	ATP dependent RNA helicase, mRNA sequence
10, 11, 12, 13	7143527	nematode specific
14 & 15	7143602	protein serine-threonine phosphatase 1, catalytic subunit
16 & 17	7143612	40S ribosomal protein S4
18	7143666	cytochrome p450
19, 20, 21, 22	7143675	Neuroendocrine protein 7B2
23, 24, 25	7143839	nematode specific
26	7143863	40S ribosomal protein S17
27 & 28	7144016	vacuolar ATP synthase subunit G
29	7144025	malate dehydrogenase
30 & 31	7144060	J2 pcDNAII Globodera rostochiensis cDNA similar to Bystin, mRNA sequence
32 & 33	7144225	similar to arginine kinase
34	7144354	pyrroline-5-carboxylate reductase

SEQ ID NO:	<u>APPENDIX 1 (cont.)</u> INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
35, 36, 37, 38	C10	ribosomal protein L18a
39, 40, 41, 42, 43	C118	ribosomal protein S11
44 & 45	C122	ribosomal protein L16/L10E
46 & 47	C127	FMRFamide-related neuropeptide precursor
48	C129	ADP-ribosylation factor 1
49	C130	ribosomal protein L11
50	C137	nematode specific; conserved in <i>C.elegans</i>
51 & 52	C138	ribosomal protein L7
53	C145	ADP/ATP translocase
54 & 55	C148	troponin
56 & 57	C154	calponin
58	C16	translation elongation factor EF1A
59 & 60	C18	40S ribosomal protein S16
61	C27	ubiquitin
62 & 63	C46	nematode specific
64, 65, 66	C48	ribosomal protein S3AE
67	C59	40S ribosomal protein S5/S7



SEQ ID NO:	<u>APPENDIX 1 (cont.)</u> INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
68	C8	glyceraldehyde 3-phosphate dehydrogenase
69 & 70	C82	60S ribosomal protein l30/L7E
71	C90	glyceraldehyde 3-phosphate dehydrogenase
72	C135	nematode specific
73 & 74	C206	predicted troponin
75	C227	cytochrome P450
76	C238	vacuolar ATP synthase subunit G
77	C246	40S ribosomal protein S4
78	C308	FMRFamide-like neuropeptide precursor
79	C342	ubiquitin
80 & 81	C344	nematode specific; conserved in <i>C.elegans</i>
82, 83, 84, 85	C370	40S ribosomal protein S5/S7
86	C426	nematode specific
87	C458	histone H4
88 & 89	C481	ribosomal protein L30E
90 & 91	C556	nematode specific; conserved in <i>C.elegans</i>

SEQ ID NO:	<u>APPENDIX 1 (cont.)</u> INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
92	C628	ribosomal protein S17E
93 & 94	C665	malate dehydrogenase
95 & 96	C669	malate dehydrogenase
97	C694	ribosomal protein S3AE
98 & 99	C709	ADP/ATP translocase
100 & 101	C714	ADP-ribosylation factor 1
102	C721	calponin
103 & 104	C726	ribosomal protein L11
105	C736	nematode specific
106 & 107	C773	troponin
108	C834	nematode specific
109	C860	bystin
110 & 111	C863	troponin
112 & 113	C883	translation elongation factor eEF-1A
116	C888	40S ribosomal protein S16
117	C898	glyceraldehyde 3-phosphate dehydrogenase
118 & 119	C935	peptidyl-glycine alpha-amidating monooxygenase
120 & 121	C937	calponin
122 & 123	C942	peptidyl-glycine alpha-amidating monooxygenase

SEQ ID NO:	<u>APPENDIX 1 (cont.)</u> INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
124	C954	arginine kinase
125, 126, 127	C969	calponin
128 & 129	7235653	ribosomal protein L18A
130	8005381	neuroendocrine protein
131	7235496	pyrroline-5-carboxyla te reductase
132 & 133	7275710	protein phosphatase pp1-beta catalytic subunit
134	7923685	nematode specific
135	7641370	40S ribosomal protein S11
136 & 137	7923404	nematode specific
138	7797811	ATP-dependent RNA helicase
139	7143613	predicted phospholipase D

## Appendix 2:

### Exemplary genes used for RNAi vectors.

#### Promoters:

##### *Constitutive:*

##### **Super Ubiquitin from Pine**

CCCGGGAAAACCCCT CACAAATACATA AAAAAAATTCTT TATTTAATTATC AAACCTCTCCACT ACCTT  
 TCCACCAACCGTTA CAATCCTGAATG TTGGAAAAAACT AACTACATTGAT ATAAAAAACTA CATT  
 CTTCTAAATCATAT CAAAATTGTATA AATATATCCACT CAAAGGAGTCTA GAAGATCCACTT GGACA  
 AATTGCCCATAGTTG GAAAGATGTTCA CCAAGTCAACAA GATTATCAATG GAAAAATCCATC TACCA  
 AACTTACTTTCAAGA AAATCCAAGGAT TATAGAGTAAAA AATCTATGTAT ATTAAAGTCAAAA AGAAA  
 ACCAAAGTGAACAAA TATTGATGTACA AGTTTGAGAGGA TAAGACATTGGA ATCGTCTAACCA GGAGG  
 CGGAGGAATTCCCTA GACAGTTAAAAG TGGCCGGAATCC CGGTAAAAAAGA TTAAAATTTTTT TGTA  
 AGGGAGTGCTTGAAT CATGTTTTTTAT GATGGAAATAGA TTCAGCACCATC AAAAAACATTGAG GACAC  
 CTAATAATTTTGAAGT TTAACAAAAATA ACTTGGATCTAC AAAAAATCCGTAT CGGATTTTCTCT AAATA  
 TAACTAGAATTTTCA TAACTTTCAAAG CAACTCCTCCCC TAACCGTAAAAC TTTTCTACTTC ACCGT  
 TAATTACATTCTTA AGAGTAGATAAA GAAATAAGTAA ATAAAAGTATC ACAAACCAACAA TTTAT  
 TTCTTTTATTTACTT AAAAAACAAAA AGTTTATTTATT TTACTTAAATGG CATAATGACATA TCGGA  
 GATCCCTCGAACGAG AATCTTTTATCT CCCTGGTTTTGT ATTAAAAAGTAA TTTATTGTGGGG TCCAC  
 GCGGAGTTGGAATCC TACAGACGCGCT TTACATACGTCT CGAGAAGCGTGA CGGATGTGCGAC CGGAT  
 GACCTGTATAACCC ACCGACACAGCC AGCGCACAGTAT ACACGTGTCAAT TCTCTATTGGAA AATGT  
 CGTTGTTATCCCCG TGGTACGCAACC ACCGATGGTGAC AGGTCTGTCTGT GTCTGTGTCGCT AGCGG  
 GAGAAGGGTCTCATC CAACGCTATTAA ATACTCGCCTTC ACCGCGTTACTT CTCATCTTTTCT CTTGC  
 GTTGTATAATCAGTG CGATATTCTCAG AGAGCTTTTCAT TCAACCCGGG

##### **Strawberry Banding Vein Virus 1**

aagcttttcactgtgggttaatttcattaatctatccaggtgaaaacctcaaggaga  
 tctctcttctccaaaagacctctacagggcaatcaaaaactacagaaccagagttt  
 gtagtgacacagagtagaccaatctacctgagaatcacgagtaccttcttagagtggtg  
 aaaatgatgacatccttattccataccactggattgaggtaggactatccaatggaa  
 aaattccatgggacaagtcatataagaagaccgcaacagtcgagtatcttccagaga  
 taactgcactcagacctaaaaggataaaaagcagtatataatcagtgtagtaagatct  
 tcgcagattcaaagaagaagctt

##### **Strawberry Banding Vein Virus 2**

Gtttaaacacagcccaagataacagaaaagtc aaaggtgttcgaaagaccacttgt  
 gactaaggatcatttcatccataattatctggtagcacagactcatgataactgcga  
 ggaacacaagttctttacagtcgattcaaagacactttctctttacggtttcattga  
 aggagccgacccagaatatgtcagagaagcttttcactgtgggttaatttcattaat  
 ctatccaggtgaaaacctcaaggagatctctcttctccaaaagacctctacagggc  
 aatcaaaaactacagaaccagagttttagtgtagtcacagagtagaccaatctacctgag  
 aatcacgagtagcttctcttagagtggtgaaaatgatgacatccttattccataccactg  
 gattgaggtaggactatccaatggaaaaattccatgggacaagtcatataagaagac  
 cgcaacagtcgagtatcttccagagataactgcactcagacctaaaaggataaaagc  
 agtatataatcagtgtagtaagatcttcgcagattcaaagaagaagcttaactatgc  
 tgatgacaagataattctaataagcaattattcagaattaatcaaggagaaagaatt  
 aataactctttcagaatatgaagcccgctttacaagtggccagctagctatcactga  
 aaagacagcaagacaatggtgtctcgatgcaccagaaccacatctttgcagcagatg  
 tgaagcagcagagtggtccacaagacgcactcagaaaaggcatcttctaccgacac  
 agaaaaagacaaccacagctcatcatccaacatgtagactgtcggttatgcgtcggt  
 gaagataagactgaccccgagccagcactaaagaagaataatgcaagtggtcctag  
 ctccacttttagctttaataattatgtttcattattattctctgcttttgctctctat  
 ataaagagcttgatattttcatttgaaggcagaggcgaacacacacagaaacctccc  
 tgcttacaaacctgtattgttagctaaacctcttaggaggtatct

**Nematode Inducible:****Trypsin Inhibitor from Arabidopsis (clone#6598343)**

ccccgggagcaaagcaagaacaccagagaagaagaaaagcactacagagaaaaatgtg  
agcttaagcgcctctccaacaacacttctctgggagctctaaaggatgctgcaaaaagc  
cttggtggtgagacttccgcataatctccaagcatgggtttatctttagcacaca  
aactatctgacctcgacttggattttctctgcagtttgtccaactacattgaaac  
ggatatgcaggcaacatgggatcatgaggtggccatctcgtaagattaacaaagtga  
acaggtcactaaggaaaatacagacgggtactggactcgggtccaaggtgtagaaggag  
gactaaagttcgactcagcaactggcgaattcattgcagttagaccttttattcaag  
aaattgatacccaaaagggtctgtcgtctcttgataatgatgcacatgcaagaagaa  
gtcaggaggatagcctgacgatacttcatcaagctccaggaagctaaatctgtcg  
acaatgccattaagttagaggaggatataccatgaatcaagcaagaccaggtaaga  
acttctctatccataaaccatagatggagcgattagaatcttaattcattttcagtt  
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ttggttatggttcggttctatatcatcaaaccaatcgggttgggtcctaagataatta  
taaatattcaccaacaccagtggttaaacacatatcaacaaacctaaagttagataaa  
caaagagacccggg

**Arabidopsis Transmembrane Protein from Arabidopsis  
(clone#6468048)**

ccccgggaattggcactcttcttctgctgggttccaaaagaaacgaatcaatatgtgc  
aacaagaagagctccagaagcagtcattctctaaaatcttaattctaacaacagctca  
agaagaaaaaaattccatagctagagagaacacaaagtcaacagacgacgtcgtaga  
ggcacaaagtcaaacctgaatggcttaagccgaactgagtggttttgactagaccat  
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ttttgttggtctcacgttttcagctgccatttgatttcagtttgggttttcctta  
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61

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gatagggttttagtagtctatttcttttgtcaatgtctttttgatacaatgccaatcc  
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gtgaatgaaacaaaggcaacagtggaagttccagctgaagaagggtctgtgcatggga  
gttgcagttggttaacctttcccggtg

**Diaminopimelate Decarboxylase from Arabidopsis  
(clone#4159709)**

ccccgggtggcaaacctgagatataagaggggaaggtgattttcatgcaaattttttttt  
tatttttttttgaatgaatgcaaaatttattcaaaaaaaaaaacctgggtacatc  
aagtaacttcatttctgagtttttgaaaaatctaaagacaacaaaagactttacaatt  
taataaaaaaataataaaaaatactttatcactctcaacgaaattgttgatttaataa  
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aagtttttctgtttttgttttatatctatagccaagaaagtttctcagatttacaaga  
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gtgatttaattttgagacaaattaaacatcgaattaaaagtaacatttggagggttta  
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acagattgtgaatgagaccaaataatgggtcataaacgggttgggtttaaacgggca  
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aagtaagacaaagaaatacagatataagatgggtcgtagaacccagtagaggaatttc  
attttcgtggataagtggaatattaataagagaatgggtctttactctttacagtgg  
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tctcttaacgtcggcaattttataattctactttacatgacactttcaggaaaagaaaa  
ctatactcactagcagatcattaaattttctttttcttttttttgaatgaaccttagt  
tgtgggtttttatttttggtagctagaaacttcagtggtttttttttccgccaatggtag

tgctttgatgatggtccggcccg

**Peroxidase from Arabidopsis (clone#4006885)**

cccggaatcaaggaacgaaggaggatcagcgaaggatgggctatatattggagt  
tttttccgtgctgtaagtaaatgctttgtgatcttccatgaggacataaactgaaga  
ataaactcaactcattgtgttctgggtgtgtttcttctgatcagattcctcgttgcat  
ctgcacttttctgctgtgggggctttatttataaaacaagagtagagcgtgtggttaa  
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ctccatctttctttgaagaagtacagaaagtacagaaattatatcaattaaacatcaa  
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tccttagggaaaaagaagcttattcttcaaattattgttatgagtcgttaaaatatgg  
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ttctccaataagtttttacaatttttaattgtttgcatttcccttgattatttatct  
tcatcccaatttagctaataccaactccggttcttattcttccaagtccttttccat  
aaatacgttcttcttccctcttattttcatatcactcaccacaaagtcttctcattt  
cctcatcccg

**Mitochondrial Uncoupler from Arabidopsis  
(clone#4220510)**

cccggaatggtgtgagtgaggagaagaagagggaaacaaaggtattttattttagtgc  
gagttttgttttgtgacgcggttttgtctgtgttcaatgttgacgaaacgagtgaga  
gagtgctgattattaaagaaaaccctaattaagtacagaccgcggttataaaaaat  
agtcaaaaagtaggaaaacgcgtgtgtgagtgagacagagacagcccattgtttgct  
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ggcacacgccttaaccactcgccaaagcgacttgttgcctatgagttagacaaaatc  
attaaaattctctattatgatttctcatagtggtgtgtgtatattgtggatctactaa  
aaattctttgttattattactttattttgtgaattagtttgatataggtaagtacaa  
agtttaactttatttttactcaaaatttatcagatttaactgattttatattgtttcc  
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 gagaacaatagaaaacccaacttgtgctctctaggggttttctttattctctctcatc  
 tttggattttcttggtcatcattttggaagcttaccaccagcgaaaaaattataa  
 ctcccatcgattcctggcttctctctcgcctctctctgcatgtgctaaatcgccgg  
 actgatcctcactgtcacctctgttcccggg

**Stress protein from Arabidopsis (clone#6598614)**

ccccggggattaggggtttgagttgtcactggaaagagggtttgattgtgagtgatgat  
 ggagagattatgaaggagtttggtgtgattttatagaggagttagggttttgaggttt  
 gatgagaagtaggtttgaagaagttttggtgttgcaacttatttagagttacttggt  
 ccacaaccacaagtaagattgggtcacttctaagttctaactagaaacaacctatgaca  
 catggagattttcagctaacctagtttaattgtatatgtattatattttatttaaatat  
 tataaaataaaacataaattttcacaaataaaagaactacaaaaaagtgaaaaaataa  
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 ctaatatattttggttggtcaaccaacaaatagatttaatttaattatgggtttatgagct  
 tttattttggttgcgacagtatatatatgtttaaatagtgatattgcatggcggaagggt  
 ccggaagcaacacatatatccttttttaatttttttttttaacaagaataacatgttaa  
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 ttttgatgggttcctgcacaatgtgtgctgctccttcgccaagacatgtaccgattcc  
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 aacaagaagtaaaagagttttcttattttccaattgttttttaattctgatacttttttc  
 ataattttacaatgtttgatgaaaaaaaacattcaaacctaaattttctttttttgg  
 tatgaattcaaacctgaattacttttgacgaggaccgacgggtataaatagggtgat  
 ctcccaacaaacaaaaagggtcccggg

**Pectinacetylsterase from Arabidopsis**

(clone#6671954)

ccccgggtgggtggggacaatggatccggtctgcgtagcaacaaggctgaaaaagatta



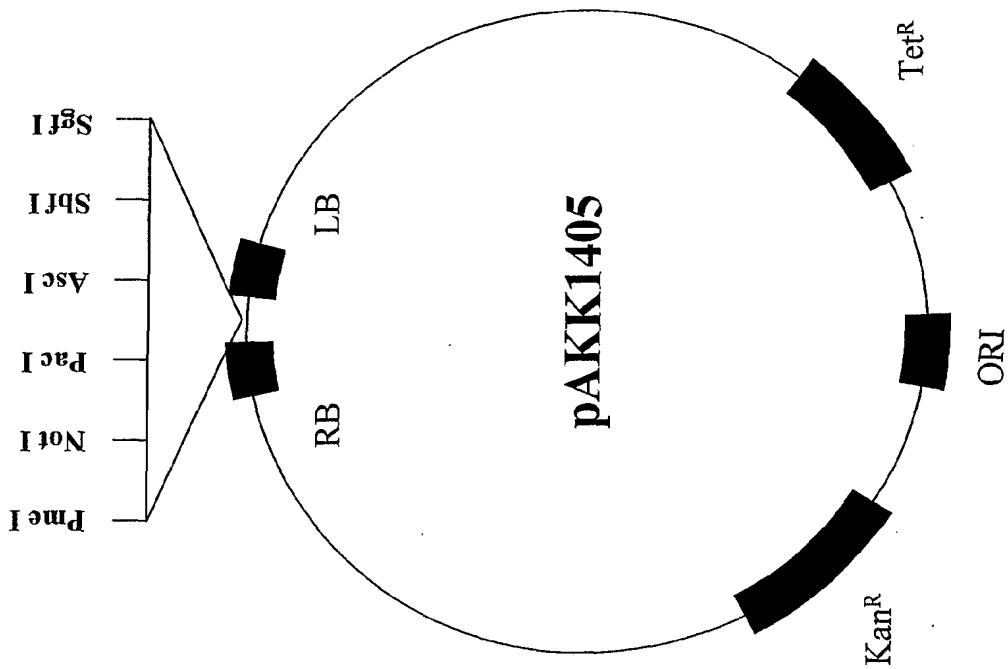


FIG. 1

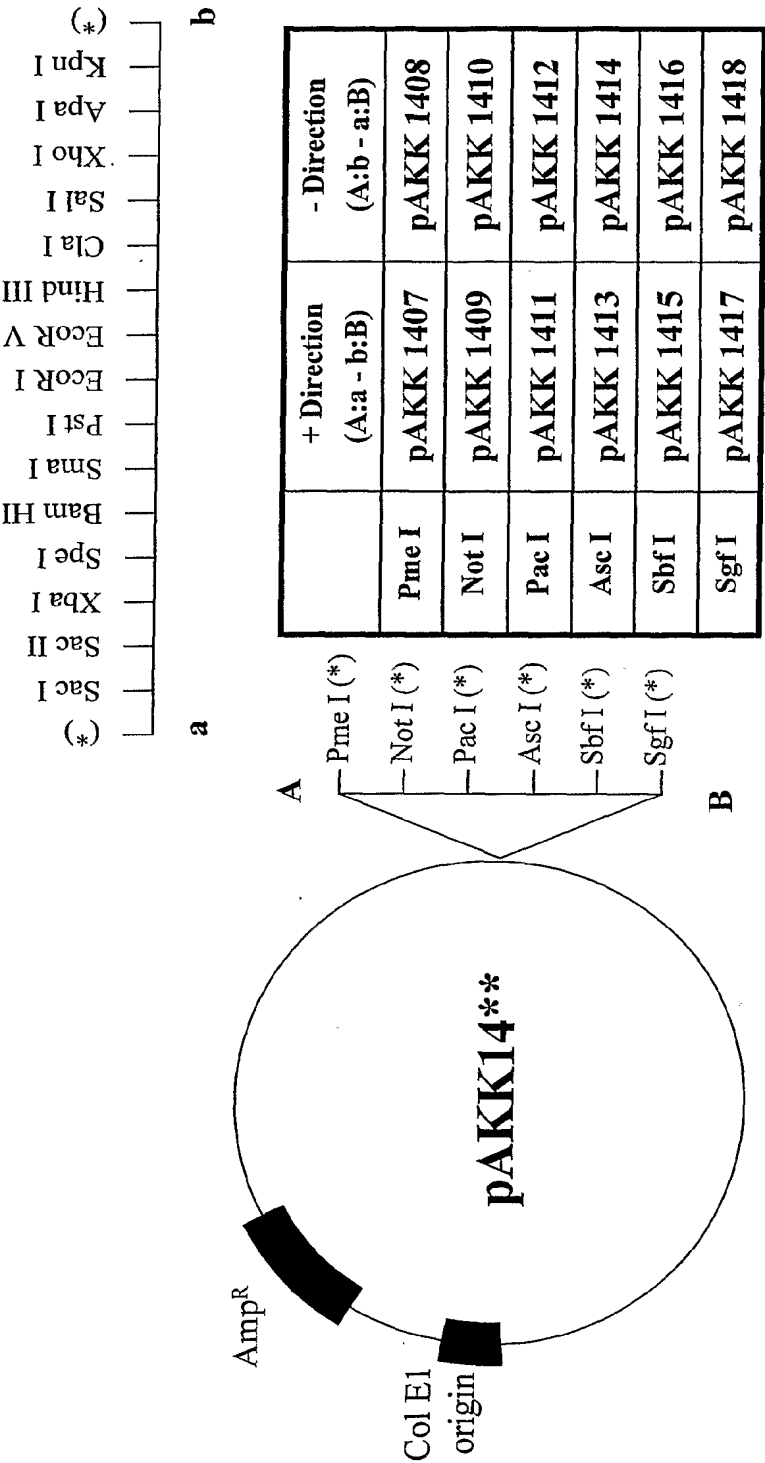


FIG. 2

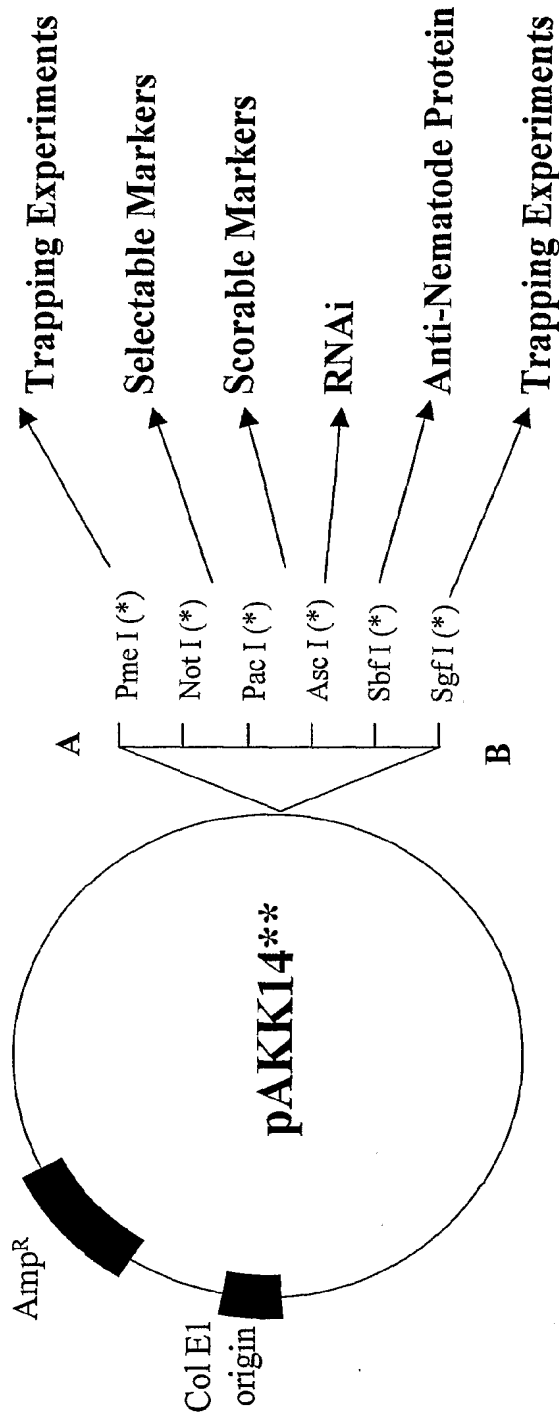


FIG. 3

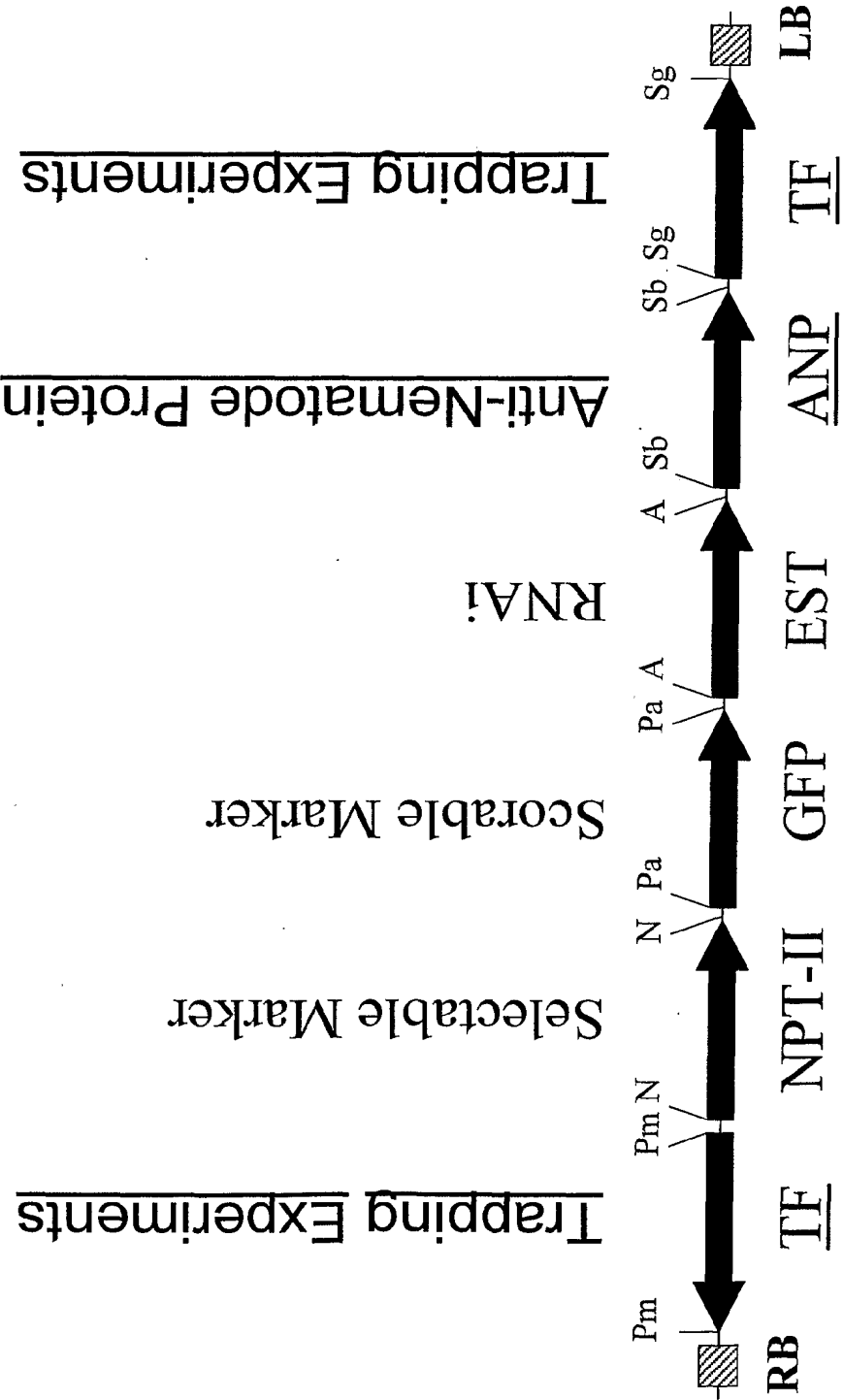


FIG. 4

Selectable Markers

pNOS / NPT-II / tNOS

pSU / Bar / tNOS

pSU/ Intron / Bar / tNOS

pUBQ3 / Intron / PMI / tNOS

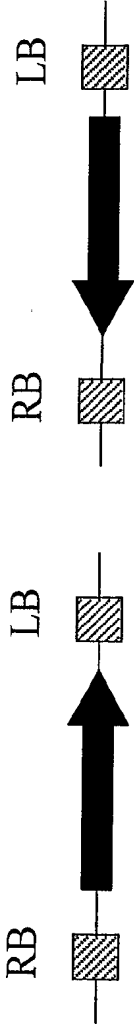
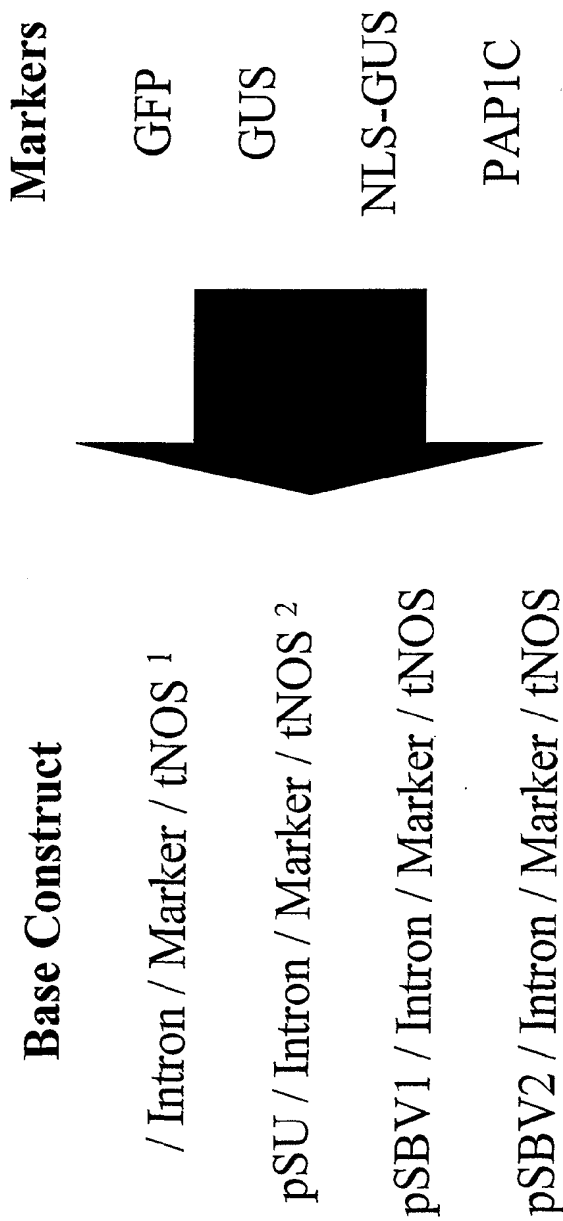


FIG. 5

# Scorable Markers



<sup>1</sup> Construct useful for promoter analysis.

<sup>2</sup> Construct useful for high constitutive expression of genes of interest.

FIG. 6

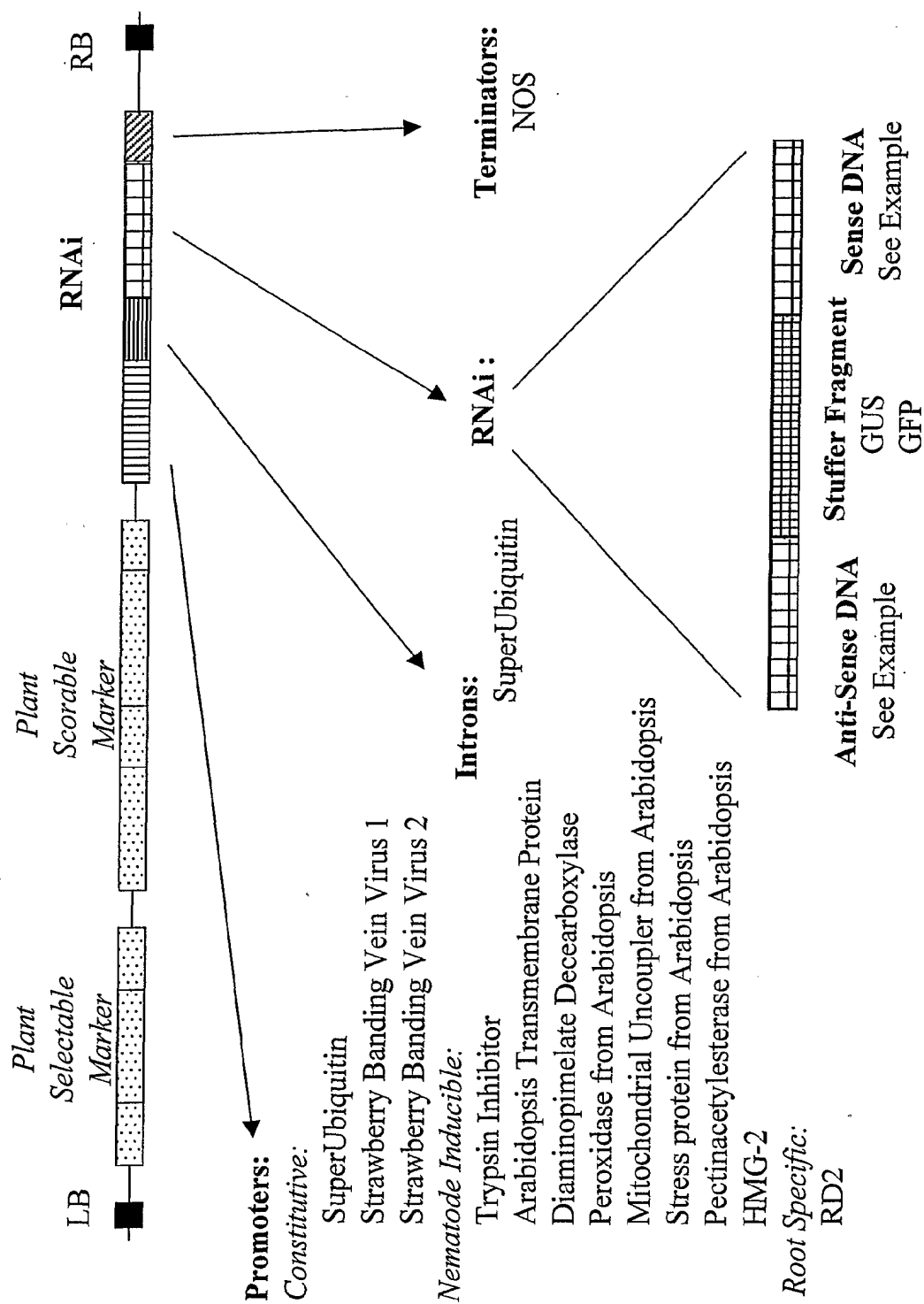


FIG. 7

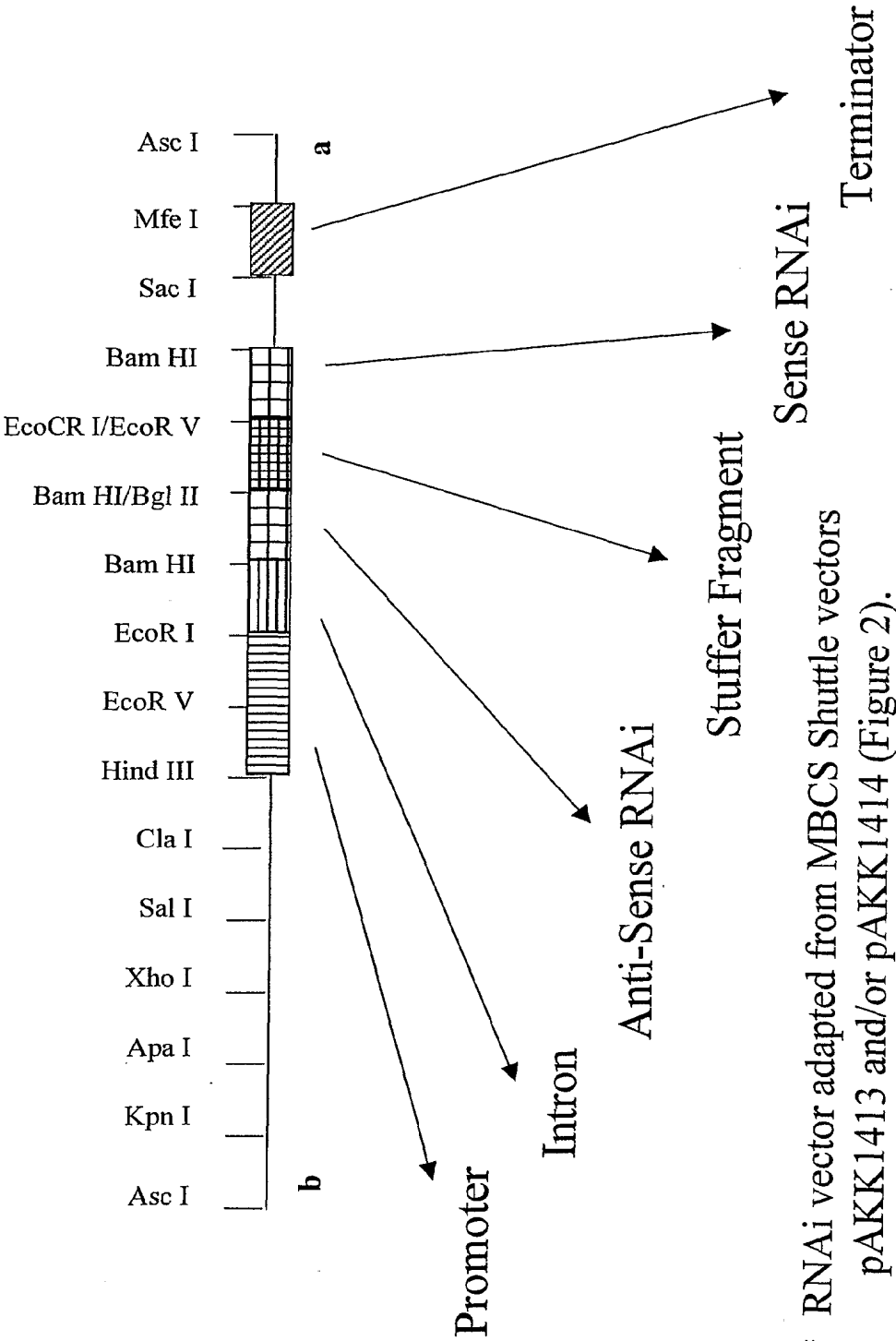


FIG. 8



AKK110P1  
SEQUENCE LISTING

<110> Mushegian, Arcady R.  
Taylor, Christopher G.  
Feitelson, Gerald S.  
Eroshkin, Alexey M.

<120> Materials and Methods for RNAi Control of Nematodes

<130> AKK-110P

<140>

<141>

<160> 139

<170> PatentIn Ver. 2.1

<210> 1

<211> 165

<212> DNA

<213> Globodera rostochiensis

<400> 1

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taaacaatagc	aaaaatggtg	aaaccgaagg	tcggcattaa	tggcttttga	cgcattgggc	120
gcttggcgtt	gcgcgctgcg	gttgagaagg	acaccgttca	ggtgg		165

<210> 2

<211> 342

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<213> Globodera rostochiensis

<400> 2

cgactacatg	gtatacatgt	tcaactacga	ctcgacccat	ggccgcttca	atggcaaaaat	60
ttcgacaagc	gccggcaatt	tggtcgttga	gaaagagggg	aaggccacgc	acaccatcaa	120
ggtgttcaac	ctcaaggacc	cggccgagat	caaatgggct	gaggtgggcg	cggaatatgt	180
gatcgagtcc	accgggggtg	tcactaccat	tgagaaggct	tcggcacact	tgaagggggg	240
cgccaagaag	gtggtcatct	ctgctccgtc	cgctgatgca	ccgatgtacg	tgatgggctg	300
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<210> 3

<211> 205

<212> DNA

<213> Globodera rostochiensis

<400> 3

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gaagggcatt	ttgggttaca	cagaggacca	ggtggtgtcc	acggactttc	ttggagacag	120
tcgctcgtcg	atcttcgacg	ctggggcggtg	catctcgttg	aaccgcgact	ttgtcaagtt	180
ggtcagctgg	tacgacaatg	aattt				205

<210> 4

<211> 167

<212> DNA

<213> Globodera rostochiensis

<400> 4

ttaaagcatt	tattcacacg	cacggagaaa	tgaggattac	ctaatttgat	tgagtctttc	60
tcgtccattt	gtcaattgtg	gccctaaaga	gggccggttg	ggttagtttt	ttggtgttcc	120
ttctccttgc	tggctcaacc	accgaagccg	tacagcgtcc	ggccttg		167

<210> 5

## AKK110P1

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 <213> Globodera rostochiensis

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<210> 6  
 <211> 79  
 <212> DNA  
 <213> Globodera rostochiensis

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 cttaacgcct ccacgacgg 79

<210> 7  
 <211> 168  
 <212> DNA  
 <213> Globodera rostochiensis

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 <213> Globodera rostochiensis

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 gccgataaag aaagacgaag aggtattggc taagaacacg ccgcacattg tcgtcggaac 180  
 gccgggacgt cttttggcct taggacgcac tggacatctg aagctgaaag gcgtcaaata 240  
 ctttgtgctg gacgaatgcg acaaaatgat tggagatgcc gacatgcgcc acgacgtgca 300  
 ggaaatcttc aaaatgacgc ctcaggagaa 330

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 <212> DNA  
 <213> Globodera rostochiensis

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 tacgtcgacg acgaggctaa gcttacgctt cacggtctcc aacaatacta cgttagactg 120  
 aaggaaaatg agaaga 136

<210> 10  
 <211> 141  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 10  
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 ttgttggtca tcaactttct cagcagcgac aatacggcca atccggtgaa agggccaaag 120  
 tcaatagctc gtcggtacc t 141

<210> 11  
 <211> 141  
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AKK110P1

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 11

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gccattttgt ttctacagca cagcacacc gtcgtcttta cagcggtcac ctcgcaaaa 120
aagtagccgt atttgcgaaa t 141

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&lt;210&gt; 12

&lt;211&gt; 37

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 12

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gcgttggtg caagctgtac acaaggctgc ccggttt 37

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&lt;210&gt; 13

&lt;211&gt; 161

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 13

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gcgcgttcca tcgcccgcac cacaaaaagt cccatcgctt catatcgtag cgcaaatgt 60
ctttggtgca aatggcaaaa cggccaaaat aatggtcgaa gccgtacaca accgccaccg 120
ccacagcgcc aacccacac caaatgcgaa atttattcgaa a 161

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&lt;210&gt; 14

&lt;211&gt; 306

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 14

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gaattcgttt gaggtataaa taaataataa atggcagcca acgaatcgct aaatgtggac 60
agtttgatca ctcgattgtt agaagttcgg gggtgtagac cgggaaaaac agtgcaaatg 120
gacgaatctg agatacgcac tttgtgcac aaaacacgtg aaattttgct gtcgcagcca 180
atcttggttg agctcgaggc accittaaaa atttgtggtg acattcacgg acaatataat 240
gatcttctga gattgttcga atatggtggg tttccaccgg aagcgaacta tctatttctt 300
ggggac 306

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&lt;210&gt; 15

&lt;211&gt; 261

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 15

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gcaaagcctt gagacgattt gtttgcctgt tgcttacaag attaaatatt ctgaaaattt 60
ttttcttctt cgtggcaatc acgaatgtgc ttcaatcaat cggatttacg gattttatga 120
tgaatgcaaa cggagggttc tcaatcaagt tgtggaagac cttactgac tgcttcaact 180
gtctgccaat tgccgcttta atcgacgaaa agatcttttg ctgccacgga ggctgtctcc 240
tgatttgcta aacatggcag c 261

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&lt;210&gt; 16

&lt;211&gt; 151

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 16

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gaattctttg agtgcattca gcgtttaatt ttttcgtatt ataataagca tggctcgcgg 60
acccaaaaag catttgaagc gacttgcagc acccaaaaaa tggatgttgg acaaattggg 120
tggcgttttt gcgccacgtc cattgtgcgg a 151

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&lt;210&gt; 17

&lt;211&gt; 306

## AKK110P1

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 17

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tggacggcaa	agtgcgcacc	gagatgcgct	tcccgtgcgg	aataatggat	gtgatctcga	120
ttgagaagac	aaacgaaacg	tttcgtctgg	tgtacgatgt	gaagggccgt	tttgtcatcc	180
atcgaattca	aaagctggag	ggccagtaca	agctgtgcaa	agtgaagaag	caggccgtcg	240
gggacaagca	ggtccccctac	attgtcacac	atgacgcgcg	caccattcgc	taccggaccg	300
ctcatc						306

&lt;210&gt; 18

&lt;211&gt; 528

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 18

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ggagcaagca	gacgaccttt	cggatttggt	ttgttcgtcc	attgggttgg	agcatcggcc	120
gttcctaccg	tatacaaacg	ctgtaataaa	tgaacaatt	cgattagtca	atttgatccc	180
gttcaatctt	agccatttgg	cgcttgaaga	tatgcaaatt	ggcaatttta	ttgtgaagcg	240
tgggacacca	attgtaccgc	aggtcagcag	tgttctgttc	gacgaaaaac	tgatccgga	300
gcccgatcgg	tttttgcccg	aacgctttct	ggacgatgag	ggccgtttga	agaaaagcga	360
cgaacttatt	gcatttgggg	ttgggaaaag	gcaatgtgcc	ggcgaagctt	tggcccgaat	420
gacacttttt	ctgtttgccc	ctaatttctt	tctcgcctac	aaagttctcc	cgctccgatcc	480
actgaatcct	ccaagcctga	aaaagttggc	ggattatctg	tttacaca		528

&lt;210&gt; 19

&lt;211&gt; 335

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 19

gaattctttt	agaaagcggg	aattcgtttt	tggctataaa	atgattctgt	gggccacgat	60
tttgttgatg	gctttggaca	ttgcgttcgg	tggcaccaat	caaagtgaat	ttgatcagtc	120
ggcgccgatg	ttccccgact	cccagttcat	cgatttgatt	tcgcgcgaca	tcgaatcctt	180
ctccggccca	ttgggcgttg	gccataaatt	tatgagcggc	ggtgccggtg	agggcgctca	240
acagctaggc	cccagggggc	cctttgagca	gcggcaacag	gtgaagagtg	acaatgttct	300
ccccgcgtat	tgcgagcctc	caaatccctg	tccga			335

&lt;210&gt; 20

&lt;211&gt; 52

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 20

ggacggctgc	acggaacagt	tcgagaacac	tgccgagttt	tcgcgcagct	ac	52
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&lt;210&gt; 21

&lt;211&gt; 190

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 21

gcttgtgtga	ccaggagcac	atgttttaact	gtccgtcgaa	gaacaaccgc	gaggagtacg	60
agcaggatct	ggagcaattg	ctggccaaca	acggactgca	caaataaatg	attgccaaga	120
aattccatct	cacgcggggc	gaggagccgc	gccgtcgaaa	acgctcttgt	cgcccggctt	180
cggccaaccg						190

&lt;210&gt; 22

&lt;211&gt; 52

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

## AKK110P1

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<210> 23  
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<213> Globodera rostochiensis

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<210> 24  
<211> 77  
<212> DNA  
<213> Globodera rostochiensis

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aacagaccgg aacagca 77

<210> 25  
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acaacgtgca gcagcaacat gttgttggtc aacaacagca gcaacaacag aatttccaac 180  
aaccgccggc cctatcgtag actcacagcc accaacaaca aaaacaacca ccacaagcgt 240  
cacagtcgat gttgtcaatg aaaagtggca atgttggtcgt tgttggtccg caacaatcgc 300  
agcagcacca ctaccaacag cggacactga cgccactgaa gcacacatcc gcacacctca 360  
cgtccgatcg cttcgtcatc accaaaacca acaggggtgct tccactcccg tcgcagcaag 420  
gcgccacggc cactgatga 439

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<211> 539  
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<213> Globodera rostochiensis

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cctcgacttt cacaccaaca agcgcatttg cgaggagggt gccattatcc caagcaaacg 180  
gatgcggaac cgaattgcgg gatttatcac acatctgat aagcgcattg agctgggccc 240  
tgtccgtggc atttccatca aattgcagga ggaggagcgc gagcgtcgcg acaattacat 300  
gcccgaatc tcttacctgg atgcgcagaa tcaccagatg atcagcaccg accaagagac 360  
gaaggatatg gcggaatttc tggggctagg cctcaacttg gaagtgaag ggcctttgac 420  
gagtggcggc gctggcgtag gacgtcgttg agtcaggaca attggcatta ttgttgaaaa 480  
atcatcgatg tttgttcgc atttgatga taatgcgctg ataaattttt gttgatttt 539

<210> 27  
<211> 179  
<212> DNA  
<213> Globodera rostochiensis

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cggccgaaaa gcgtgcggca gaaaagatta atgatgccc gaagcgaaaa gcacagcgac 120  
ttaagcaggc caaacaagaa gccaggcgag agatcgagca gtatcgncag gagagggag 179

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 <211> 133  
 <212> DNA  
 <213> Globodera rostochiensis

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 gtcgctggag gcaatgaatc gcaatgtcgc ggcgaacaaa cagcagggtca ttgtacgtct 120  
 gctgcagttg gtg 133

<210> 29  
 <211> 482  
 <212> DNA  
 <213> Globodera rostochiensis

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 caaaaggcga tgtgtttggc aaagatcagc caattgttct cgttctctc gacattccac 180  
 cgatggccga agtactctct ggtgtccatt ttgaattgat ggactgtgcg ttggcaaac 240  
 ttgccggtgt ggaggctgtg accacggaag agcaggcctt caaggacatt gactacgctt 300  
 ttcttgtcgg agcgatgccc cgaagagagg gaattggaacg aaaggacctt ttggcggcaa 360  
 atgtcaaaat tttcaagtcc caaggcgaag cattggcccg cttttccaag cccgtncgtc 420  
 aaagtctctg tgggtgggcaa cccggccaac acgaacgcgt acatttgcg aaaatatgcc 480  
 gg 482

<210> 30  
 <211> 605  
 <212> DNA  
 <213> Globodera rostochiensis

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 ttttctgccca cgggcacacc gtcacaatgc caaaggttca acactttggt gctgttgcca 180  
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 tttaaagcaa tgttcaagcc ggccggattt ttttaaggga ttattttgcc tctttgcaa 300  
 tctggcactt gcaactctcg tgaagccatc atctttgggt ctgctctgcg aaagatttca 360  
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 gccatttctt ttatcctacg tgttcttggt gaaaaaaatt acacacttcc tttccgagca 480  
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 cagag 605

<210> 31  
 <211> 112  
 <212> DNA  
 <213> Globodera rostochiensis

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 aatgaggaat gtgaagcaaa tgtgcccgtt tatgcgcgta atgatgaaat gg 112

<210> 32  
 <211> 105  
 <212> DNA  
 <213> Globodera rostochiensis

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<210> 33

AKK110P1

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 <212> DNA  
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 gactaccatg gtggcttttg tccgggcagc aagcagccgg caactgacct tggtagacggc 240  
 aaaacgcana tgctgaccgg atctcgaccc cgaggggaaa atttatcaat ttcgacacgc 300  
 gttcggttgcg gccgtttcct ttaagggata cccggttcaa cccgtgcttg acnaaaggan 360  
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 atcct 425

<210> 34  
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 tttgacggtc attccaagca gccaaataaac caccaaaacc aaataacccc cccaatcga 180  
 tccccccct ccaattcctc cgcattattc gcattatcaa ttctaatacag cacaaccact 240  
 gcatcattcc tttcccgaac atacgatgct aagtgaact ttgaaaattg gcttcacg 300  
 agccggaaag atggcccaag cattggcaag aggacttatt aattcggggc gatacccg 360  
 agagaatttg atggcgagtt gtccaaagac ggacgaggct ttactggagc aatgcaaaaa 420  
 attgggaatc ggaacgacgc acgacaacac tttggtcgcg cgagagaacg acgtcatcgt 480  
 attggcggtc aagccgatgc acatcagcaa agtgacgtcg gaaatcgac ccaatttccg 540  
 gagggaaacat ttgcttattt cattgattag gaattacact t 581

<210> 35  
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 <212> DNA  
 <213> Globodera rostochiensis

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 cccatcaaag catccggaga aacattaagg aagtttattg tc 102

<210> 36  
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 <213> Globodera rostochiensis

<400> 36  
 tgcaaagat gcaaaccaca cgcttcacaa gatg 34

<210> 37  
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 aacacacgga gagatcggtt cgtgtcaaga ggttttcgag 100

<210> 38  
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 <212> DNA  
 <213> Globodera rostochiensis

<400> 38

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 gctcgtcacc gcgctcaggc ggatcgaatt caaatcatca aagtgcaaac ctcaag 176

<210> 39  
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 <213> Globodera rostochiensis

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 ttgggaagaa ggcattccaac aaggaccgtt actgg 155

<210> 40  
 <211> 35  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 40  
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<210> 41  
 <211> 70  
 <212> DNA  
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<400> 41  
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 gcggacgatt 70

<210> 42  
 <211> 85  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 42  
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 cgtgcttcgc agatgtctct ctcgg 85

<210> 43  
 <211> 193  
 <212> DNA  
 <213> Globodera rostochiensis

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 attctgagtc ggccaagcca accgcgaacg gtcatttgtt atgggttccta attgttgctg 120  
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 tgaaaaagct cga 193

<210> 44  
 <211> 219  
 <212> DNA  
 <213> Globodera rostochiensis

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 gaagacgtcc ggcgcgttgt tatcgctata ttaagaacaa gccgtatccg aagtcgcgct 120  
 tttgtcgcgg tgtacccgac ccaaaaattc gcatttttga ttggggtaga aagcgcgcga 180  
 ccgttgacga attcccatgc tgcgtgcata tgatatcga 219



AKK110P1

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 <212> DNA  
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 atgttgctct gcgctgggtgc ggaccgtctg cagactggga tgcgtgggtgc gttcggaaaag 180  
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 gaccaacacc aagctcacgc attggaggcg ttccgtcggg cttaaattcaa gttccctggt 300  
 cgtcaataca tctgtcttgc cgcgaagtgg ggcttcacca aattcgatcg cgaggatatac 360  
 gagaaatacc gcaaggaggg ccgtgttatc cctgacggtg tgcattgcaa gttactcaag 420  
 caacacggac ccgctgaagg agtggctcaa gaacccatt taatcttctg tttgtcttgt 480  
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<210> 46  
 <211> 101  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 46  
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<210> 47  
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 <213> *Globodera rostochiensis*

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 accgcgccgt ttattttgtg ttcccagaaa acttgccgtt ggagcggccc ttcgacgagc 180  
 aaaacgacgg ctccgaggag gaattagccg aagaagcgat gggaacgaag gcgaagaggg 240  
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 cacaacatt tgtacgcctc ggaagggaca cgcaaaggca attcgatggg aaaatgcaaa 360  
 gtgaacagca acagaaaaag gcttaaagca aacggcggcg acttttcttt taatgaatgc 420  
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 taaca 485

<210> 48  
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 <212> DNA  
 <213> *Globodera rostochiensis*

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 caacaattgg cttcaacgtg gaaaccgtcg aatacagaaa catctcgttc actgtttggg 180  
 acgtgggtgg tcaagacaaa attcgtccac tttggaggca ctacttccag aacacgcaag 240  
 gactgatctt cgtcgtggac agcaacgac gcgagcgtgt gggcgaggcg cgtgaagagt 300  
 tgaatgcgaat gctggcggag gacgagttgc gcgacgcggt gttgctgggtt ttcgctaaca 360  
 aacaggattt gccgaatgcg atgaacgccg ccgaactgac agacagactt ggactgcaca 420  
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 ctgttgcaat tgcggcggga attgatgacg attgaattta tttgtgtgtt tgcgcgcgca 600  
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 <211> 660  
 <212> DNA  
 <213> *Globodera rostochiensis*

## AKK110P1

<400> 49  
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 cccaactgag atcaaaatcg tgtacctgcg ttgctgcggt ggtgaaattg gtgcaacatc 120  
 tgcacttgca ccaaaagtgg gccacttgg attgtcgccc aaaaaaattg gtgaagacat 180  
 tgcgaaggcc acacaggact ggaaagggct taagggtacc tgcaagctga caattcagaa 240  
 tcgtgtcgcc aagatcgacg ttgtcccatc ggccgcctct ctgatcatca aagagttgcg 300  
 cgaacctccg cgagaccgca aaaaagtcaa aaacgtgaag cacaatggca acctgaccat 360  
 cgagcaagtg atcaacattg cgcgtcagat gcgcccctcg tcaatcgcac ggaagttgca 420  
 gggcaccgtg aaggaaattt tgggaaccgc ccagtcggtt ggctgcacca tcgatggaca 480  
 acatccgcac gacattgtgg acgcatcag agggggagac atcgaaatac ccgaggaata 540  
 aagaaaggac ggcgcctccg atttttgtgg gacggacatt gggaatttga ggtgaatgag 600  
 ttgccaatth cattcattca tcaattgttg ttattgntgg tacggataaa tttgtaattg 660

<210> 50  
 <211> 625  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 50  
 gtgccggaac agacgctcga ggaggttagc cgtctgcagc ggacgagctc cttgttggac 60  
 gtggcaatcc gggacggcgt cccctacccc ccactgcctc ctacaaaccg atccccgaa 120  
 tacatgaaca tgctgaccgg ctcttctctc gtgccaaatt tccgcatcta ctccggcgcc 180  
 atcggaccgt acagaccttc gttgcccgtg tacacttaca acacttacca cgggtacttc 240  
 ccttaccgca actaccgagg ctacaccttg gcgaatgctt actggtacga ccgatactat 300  
 tacttctcgc cgctgtacaa acgaagcatg ttccccaccg gcttcaaaca ttgtgactat 360  
 aaagcgaacc cgcactattg gcactacccg cacacctttt gggactatcc ctaccagggc 420  
 aaatggttcg actacgacaa ccctcccaat taccggccct actacaacca tcgccttaac 480  
 ggatatgctc ggccgtatca ctaccggtcc catgcgctgg cccaccggtt caattaccgg 540  
 gaaggaaattg tcaggaaacg ggtctgacaa atcgaactgc tccaaattga cgtggtccgc 600  
 attcgaaaga agacgaaaaa agctt 625

<210> 51  
 <211> 402  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 51  
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 cggaaacttt gctcaagcga cgcaaaatca gagctgcgca aaaggccgca aaagcaaaga 120  
 acaaatgtgag ttctatcaaa aaagcacgga ccaagaagggt ggaaatcttc aaaagagccg 180  
 agcagtattt ggtggagtac cgtcagaagc aacgccaat gcttgcgctg aaacgtgaat 240  
 cgaagaaagt cggcaattat tatgtgccag aagagcccaa actcgccttt gtggtccgaa 300  
 tcaaaggcat caataagatt catccgcgtc ctgcgaagggt tctgcagctt ctccgcttgc 360  
 gtcagatcaa caacggcgtt ttcgtaaagt tgaacaaggc ga 402

<210> 52  
 <211> 433  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 52  
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 aacgcggtta cgccaaagag aaggacagc gcattccaat aacggataac aacattgttg 120  
 agcgcagttt gggcaagcat gacgtgattt gtgtggagga tatgatccat cagatttggg 180  
 ccggtcggag cgcacttcaa acaggtgacc aacttcctat ggcctttcaa gctgagcaac 240  
 ccggtggggc ggttcaagaa gaagtccaat cacttttgtg gagggaggcg attatggaaa 300  
 ccgcgaggac caaatcaaca aattattgga aagaatggc taatggaagg gaagcggana 360  
 aagaaaggaa attgnggcgt ttttctgttg ttgttttgac gataaattgt taactccaaa 420  
 aaaaaaaaaa aaa 433

<210> 53  
 <211> 768  
 <212> DNA

AKK110P1

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 53

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gaattcgttt gaggtcaaac tttattagcg tatattaaca tgtccgaagg aggagcgaaa 60
aagagtagca gcggtgccaa ggggggggtt gatgtcaaga aatttgcatg cgatcttgcg 120
tccggtggta ctgcggcggc tgtctccaaa actggtgttg ctccattga acgtgtcaaa 180
ctcttggtgc aggtgcaaga tgcttccgct cacatcactg cgcacaaacg ctacaaaggc 240
attattgacg tgcttgctcg tgtgccgaaa gagcagggct ttctgtcact gtggcggtgg 300
aacttgcca acgttatccg ttatttcccg actcaagcgc tgaacttcgc ctcaaagac 360
acctacaaac gcatctttac ggagggactg gacaaaaaca agcagttctg gtcgttcttc 420
gtcatgaatt tggcctctgg aggtgcggcc ggcgccacgt cgctgacctt tgtttatccg 480
ctgggacttt gcccgctacg gtttggcccg tcgatgtccg aaaagctggg tcccgcgagt 540
tcaacggttt ggcccactgc atcgcaaaaa tcttcaagtc ggacgggtccc atcgggtctt 600
accgcggtt cttcgtctcc gtccagggca tcatcattta ccgcgccgcc tactttggat 660
gctttgacac cgcaagatg attttcgcgc cggatggcaa gcagatgaat ttcttcctca 720
catgggccat cgctcaggtc gtcaccgtgt cgtccggtgt cctctcct 768

```

&lt;210&gt; 54

&lt;211&gt; 338

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 54

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gaattccagc agattaattg gaatggctga gaacatcgaa gagattcttg ccgaaatcga 60
cggctcccaa attgaggagt atcaacgctt tttcgacatg ttcgaccgcg gaaagaatgg 120
ttacattatg gccacccaaa ttggacaaat tatgaacgcy atggagcagg actttgacga 180
aaagaccctc cgaatttga tccgcaagtt cgacgcggac ggttccggca aactggagtt 240
cgacgagttc tgcgcgttgg tgtacacggt ggccaacact gtggacaagg acactctgcg 300
aaaggagctg aaggaggcat tccgactctt tgacaagg 338

```

&lt;210&gt; 55

&lt;211&gt; 267

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 55

```

gaaattgcgc ccgatctcag cgacaaggat ttggaggcgg cggtcgacga aattgacgag 60
gacggcagcg ggaagatcga attcgaggag ttctgggagt tgatggcggg cgaaccgac 120
tgagaaaaga gcaaatcgat ccaaatccaa acggaccctg cccatttcac ctccatccgt 180
ccgtcgtatt attatatatt ccagtggaa tttccatta aaattcgggt aaagtaaaat 240
aatttgacga aaaaaaaaaa aaaaaaa 267

```

&lt;210&gt; 56

&lt;211&gt; 597

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 56

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gaattcgctg gacacttcgc atccggagta cagccacgag cagagcatcg accagaccag 60
catcccctac cagatgggtt cgaacaagta cgcctcgag aagggcatga ccggctttgg 120
acagcccctg tgggaggtgc ttgacctgc catctcgtac cagaaccgca agtcgcaagg 180
aatgggttcgt ctacagtcgg gtaccaaccg gttcgctcc caggcgggca tgaccggctt 240
cggcacaccc aggaacacca cctatgaggc ggaggcaggc gagctgccct acgaggacat 300
gaagaagtcg gaggcgatca tcccgtccca ggcgggttg aacaagggcg actcgacaga 360
gttgatgacc aacttcggca cgccccgtaa caccaccacc aaggtcaaa gggagaaatt 420
ggcggaaaatt ccggaggaca ttttctgtaa aggacacggc gaggtgcgcc tgcagtccgg 480
taccacccgg ttcgcgtccc agaagggtt cgtcgcgttc ggtaccggac gtgacgtgtg 540
ccgtgagggg gtgaacgtga acgtgctgcc gggcgacttg gagccgcttc cggagga 597

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&lt;210&gt; 57

&lt;211&gt; 80

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

## AKK110P1

<400> 57  
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 ttcggtacgg gcccgtcgtg 80

<210> 58  
 <211> 513  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 58  
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 gncggtctgg caagaaagt gaggacaacc cgaagtcgct gaagactggc gacgccggaa 120  
 ttgtcgaact gattccgacc aagccgatgt gtgtggaggc attcactgac tacgcaccgc 180  
 tcggccggtt tgctgttcgc gacatgaggc anactgttgc cgtgggacgc atcaaatacag 240  
 tggagaagac ggaaggcggg ggcaaagtga ccaagccagc gcagaaggtc ggcgcgactg 300  
 gtggcgggaa gaagacatga ccaaggggag gggcggttcc ctaagggccca accgtcgacg 360  
 aaaaatgcgc caacctcttg tttatcgttg tcttattcag ttccttccac ccgtctctat 420  
 ccatattgtc gttgcgttgg ataattgttt attttttgtt attgtcctgg ttggaaaata 480  
 aatttggtca attaaaaaaa aactcgtgcc gaa 513

<210> 59  
 <211> 393  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 59  
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 gttcaacagc ccgtgcaggc ctttggccga aagaagacag caacagccgt tgcgtttgca 120  
 aaaaggggca agggcttgat caagggtcaat gggcgctcct tggactacat gcagccggag 180  
 attctgcgca ttaagctcca ggagccaatt ctcatgttgg ggaaggacaa atttgagggg 240  
 atcgacatac gaattccgct caagggcggg ggacacattg cgcaaattta tgcaattcgc 300  
 caagcactgg ccaaggcact ggtcgctttc taccagaaga atgtcgacga gcagagcaaa 360  
 aaggaactga aggagcaatt tgttgcttac gac 393

<210> 60  
 <211> 154  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 60  
 cagagccaa agaaattcgg tggacccggg agctcgcgct cgctaccaga atcgtaccgt 60  
 taagaaataa tttttagat caaatgtttt gatgatgatc cttgtttttg ttgttgataa 120  
 aaaaaattta taaaaaaaaa ccgccgatac tgac 154

<210> 61  
 <211> 666  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 61  
 gtattccaag tttgagcgat cagagttctt caatctatta tcaactgttt tccatcaacc 60  
 aactgtcatc atgcaaattt tcgtcaagac gtcaccggc aagaccatca ctctcgaggt 120  
 cgaggctagc gataccatcg agaacgtgaa agccaagatc caggacaagg agggcattcc 180  
 gcctgatcag cagcgtctga tcttcgccgg aaaacagctt gaagacggac gcaccttggc 240  
 cgactacaac atccagaagg agtccactct ccactctcgt ctgctctcc gtggcggaat 300  
 gcaaattttc gtcaagacgc tcaccggcaa gaccatcact ttggagggtcg aggccagcga 360  
 caccatcgag aacgtgaagg ccaagatcca ggacaaggag ggcattccgc ctgatcagca 420  
 gcgtctgatc ttcgccggaa aacagctcga agacggggcg actctggccg actacaacat 480  
 ccagaaggag tccactctcc atctcgtctt gcgtcttcgt ggaggagaga actgaatcgc 540  
 gggctgatgg aaagatgacg aatatgatgt ctattcgatg acttgtctct ttcgatataa 600  
 ttgatttgtt tccatttgtc ggtcatcaaa tctttatgac cccctcattg ggcattggaac 660  
 gataaa 666

## AKK110P1

<210> 62  
 <211> 213  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 62  
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 agcactcaac cacgggacgc gtgtactgag cgtgttggag aagggtcaagt tgggtctgctg 120  
 gtttgaggag acacattcgt tcgcgcaagt ggctcgaaga taccgggcag aatttggtat 180  
 ggaaccaccg cagttggacc aagtgaagaa gtt 213

<210> 63  
 <211> 488  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 63  
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 ggtcttggag aacaacagcc aattcccgtc gtaagcgatg cgggactgga tgcggaagaa 120  
 cagctgagaa tggccagaat gtgagccgga ggacctgaag atttatgaac gaaattttcc 180  
 agtgaagtgg accaacgctc ttcgacttta tctgctttgt gtaaagtgtg tagaatcggc 240  
 ttccaattca aaggcttttc attccccaac ttttattttt gcgcaaaaaa tttcttagga 300  
 taagcgtgaa taattttattg atttgttttt tctttctttt atctccgcct cgaagtgcga 360  
 agtggttcctt ttggcccgtt ccctttttgt ttgaatgtta ttccattccc atcccctcac 420  
 tttctcatat ttgtgacatt cagctgcatt gttcgactcc catttaaaag ttgagtgaag 480  
 tgcgattg 488

<210> 64  
 <211> 249  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 64  
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 gkgdyrbwnt msnwrmanrg artsstsgaa ttcccaagtt tgagagtaaa tattatttagc 120  
 taaaaatggc agtcggaaag aataagagaa tgggcaaaaa gggagccaag aagaaggctg 180  
 tcgatccgtt cacacgcaa gaatggtacg acatcaaagc gccggcgatg ttcaacacatc 240  
 gaaatssts 249

<210> 65  
 <211> 362  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 65  
 wcbcrbhdby ytsgcrsnck tbdshbcysy gcdwkmtnvk hscngdckty nyykkkvbmr 60  
 ntmsnwrman rgartsstsg tcaaccgtac tcagggaacg cgcatttcga gcgactttct 120  
 aaaaggccgc gtttacgaag tgtcactggg tgaccttaac agcactgacg ccgactttcg 180  
 aaagttccgc ctgatctgtg aagaggtaca gggcaagatt tgcctgacca actttcacgg 240  
 aatgtcgttc actcgggaca aactgtgctc tattgtcaag aagtggcaca cgctcattga 300  
 ggcgaatgtg gcagtgaaga ctaccgacgg tttcatgctc cgactctttt gtatcgggtss 360  
 ts 362

<210> 66  
 <211> 128  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 66  
 aatcaaatta agaagacgag ctatgcaaaa gcctctcagg tgcggatgat tcgtgccaag 60  
 atggtggaga tcatgcagaa agaggctctt tccggcgatc ttgaangaaa gtagtcaaca 120  
 agcctgat 128

## AKK110P1

<210> 67  
 <211> 502  
 <212> DNA  
 <213> *Globodera rostochiensis*

<400> 67  
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 gttcctacgg ttgacccatg ggggtgctgt gaggaagtgg gtggtgaaga gtcgatgcag 120  
 ttggtcagcc ttgacgttac cgagggtcaaa ctgttcggaa aatgggtccct taacgatgtg 180  
 gaagtgtccg acatttcgct tgtggattat attgcggtga aggaaaaggc ggccaaatat 240  
 ctgccgcaca gcgccggccg ttaccaacag aagcgcttcc gcaaggccac ctgtccggtg 300  
 gtggaacggt tgtctttgtc aatgatgatg cacgggcgga acaacggaaa gaaactaatg 360  
 gcggtgcgca ttgtgaaaca ccccttcgag atcatcacct gctaccggag agaaccag 420  
 ccaagtgttg gtcaatgctg tgataaacag tgggccccnc gaagattnca cacgtatcgg 480  
 acgtgcgggc actgttcgtc ga 502

<210> 68  
 <211> 519  
 <212> DNA  
 <213> *Meloidogyne incognita*

<400> 68  
 gcaaaccttt atcaaataaa aaatttatat ttgccaaaca aatttatgaa taaaaattca 60  
 ttaatcatta aaactacatt taaaatatac tttttagaga atgtcgtcta aaatattctt 120  
 ttctccccct tatgcatcta tctaaccaga cttggaagca atatggctaa tcaagtcaac 180  
 aatacggcag gaatacccaa actcgttatc ataccagcta accaatttaa caaatgcgg 240  
 gttgagaacc ataagagcct cggcgtcgaa aatagacgaa tgagtgtcgc caagaaagtc 300  
 ggtagaaca acctggtcct cagtatatcc aagaatccct ttaagctttc cttccgaagc 360  
 agtcttaatt gcattcttaa tagcctcctt cgttgctggc ttctccaaac gagcagtc 420  
 atcaacaacg aaaacgtttg ggcgtcggca cacgaaaagc catttcgggt aagcttccca 480  
 tccaattcat ggattgacct ttccaacagc ctttgagc 519

<210> 69  
 <211> 218  
 <212> DNA  
 <213> *Meloidogyne incognita*

<400> 69  
 ttgattcttt attagtggac aatgacggaa gaccagaaga agttgccgat ggtgcctgag 60  
 actgttttga agcgaaggaa agttagggct gctcagcgtg cttctctact caagaataaa 120  
 ttggagaata ttaagaaggc taaggttaaa acgcaagtta tctttaaacg tgctgagcaa 180  
 tacttgattg catatcgacg taagcaaaaag caagagtt 218

<210> 70  
 <211> 293  
 <212> DNA  
 <213> *Meloidogyne incognita*

<400> 70  
 taagaaagca gggaattttt atgtcccaga tgaacctaaa cttgcttttg ttgtgcgtat 60  
 taagggaatc aacaaggtta atttaaattt gctataaagt ttaggatggg tttagacaat 120  
 tcttctcttt taatgctttc taactttttc aaaaaagtta tgattttatc acccattaat 180  
 ctacaaattc tttaatttat cagatccatc ctgcgtcctg aaaagttctt caacttttcc 240  
 gcttgctgca aatcaacaat ggagttttca ttaaattgaa taaagctaca atc 293

<210> 71  
 <211> 422  
 <212> DNA  
 <213> *Meloidogyne incognita*

<400> 71  
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 caggttgttt ctaccgactt tcttggcgac actcattcgt ctattttcga cgccgaggcg 120  
 taagttttga ttttctaaga ttatatttaa ctttttaaat ttttcagtct tatgggtctc 180

## AKK110P1

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aaccgcatt ttgttaaatt ggtagctgg tatgataacg agtttgggta ttcctgccgt 240
attgttgact tgattagcca tattgcttcc aagtctgggt agatagatgc ataaagggga 300
gaaaagaata ttttagacga cattctctaa aaagtatatt ttaaattgtag ttttaattgat 360
taatgaattt ttattcataa atttgtttgg caaatataaa ttttttattt gataaaagtt 420
tg 422

```

<210> 72  
 <211> 374  
 <212> DNA  
 <213> *Meloidogyne incognita*

```

<400> 72
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tgagccagtt aaactggact tcaacgttcc gcttattagt gattgggctg ctgcttctga 120
gtggcctcaa gaagaggaag ctcagggttc acctactgca ccaattgggtc agccacagcc 180
tcaacagcag caaactcaac aaggaggtga ttggaactct ggtactagtg gatggtgaag 240
ggcaggaaaa ttgatagaaa gagaaattat tatggaataa atgtaataca tgttgtgtgc 300
tgattttatt gttacatata caacaagttt tatittgttg tttatttaat aaaagtgtgt 360
aattaaaaaa aaaa 374

```

<210> 73  
 <211> 120  
 <212> DNA  
 <213> *Meloidogyne incognita*

```

<400> 73
tttttttttt tttttcttca tcaatatttt gaagtgaaga accagaagta gttgcattcg 60
agctttcaaa ttttgttttt tgattactct ttaaacaaga ttcaactgat ggatctactg 120

```

<210> 74  
 <211> 369  
 <212> DNA  
 <213> *Meloidogyne incognita*

```

<400> 74
gtctaaccac tctagagcta ttcggttcgt ctgtctgttg attattagat gttgattgaa 60
cagcactagt ctctgatgta gttttcttca atctcatttt taagtgatgt agaggaagtt 120
tagaattctg attgctatcg tcttctttct ctctctttta tggctttttc aatttatctt 180
cttctttttc ttgtccattc ttttcttcat tcttttcaaa aggtctcagga aattttaatt 240
cagaccgctg ccttttaact gctgtatcta aagaaaaccc tctaggcaac gtcccagttc 300
cactcaaatt caattttgtt aaatttttgc cagatctaag tccttcttcc ttttgaacga 360
attgaactg 369

```

<210> 75  
 <211> 529  
 <212> DNA  
 <213> *Meloidogyne incognita*

```

<400> 75
ttttgttttt tttttttttt ttatcagaaa aaagttttaat cagaaaaaaa aattaaaaca 60
aatctaaata aggctctatt ctaagtttat atttttcttt tacataaacc gtcaaccctc 120
caagtttttc aatgcttggg ggttttaatg gatcctctgg taataatttg taggctagaa 180
aaaagtgtgc agcaaaaagg aaaagcatca ttcttgctaa ggcttctcca gcacattgcc 240
ttttcccccac accaaaagct attagctcgt cagctttttt taatttccct tcattgtcta 300
tataacgttc agggtcacaaa ttttggggat ttgggtatat ctttggatca aaaagaacat 360
ccgatacttg gggatcaca aatgtacct taggcaacac aaactttcca acattcaaat 420
cttccaaggc taaatgcccc aaattgaaag ggactaaatt aacgagtctt aatgtttcat 480
taacaacagc atttgtataa attaatatag gtctgtgttc caaactaat 529

```

<210> 76  
 <211> 449  
 <212> DNA  
 <213> *Meloidogyne incognita*

## AKK110P1

<400> 76  
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 ttcaacaatt acttgcagca gaaaagcgtg ctgcagaaaa gattaatgag gcacgtaaaa 120  
 gaaaggcaca acgacttaaa caagcaaaac aggaagcgca agctgaaatt gacaaatata 180  
 gagaggaacg tgaaaaacgt tttaaagagt ttgaacataa ttacctcggc gctagagatg 240  
 atatttctgc acaataaaag cgtgaaactg atgagacgct taatgaaatg actcgtagtg 300  
 ttgctgctaa taaacagcag gtaattgttc gtctacttca acttgtctgt gacattcgtc 360  
 cagaactgca tcacaattta caacttcaac ttaagcttaa tgaaaagcct gcctaatttg 420  
 tagttgattg attataaaaa tgaaattga 449

<210> 77  
 <211> 643  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 77  
 atttatattt gaacaaataa tttacaataa aagtattggct cgaggaccac agaagcattt 60  
 gaagcgtttg gccgctccaa agaattggat gttggacaaa ttgggtggag tttttgcccc 120  
 acgtcccatg tgcgggcctc acaagcttcg tgaatcgctt cctcttattt tgtttcttcg 180  
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 tccggatcca cacatcaagg ttgacgacac tgttgctgtt gatataaaca ctggaaaggt 540  
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<210> 78  
 <211> 584  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 78  
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 aaataataaa ttggaatata ataaaaatga aattgagagg caaaaagagc aattaattcg 180  
 agatttgatt gccctcttaa cagctgaaag gcaatattca cgagattggc aacaatcaca 240  
 acagcaacaa aatttcatta acagtttttg cccttcccca catttattcc cctcttcagg 300  
 cattgaatgg ccccaacaac aacaaaaaat atttttggaa gaaggggaag tagaagaacc 360  
 tttagaggaa aatgagaagg aaaaaagagc acaaaccttt gttcgtttcg gaaagagagc 420  
 acaaacattt gttcgttttg gaaaaagggg acagactttt gttcgatttg ggagagattc 480  
 aaaacatcaa cataacttgt cagatcagaa gcagttaaaa actgacaaac aataaaaatg 540  
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<210> 79  
 <211> 556  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 79  
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 tcccgcctga tcaacagcgt ttgatctttg ctggttaagca acttgaagat ggacgaacct 180  
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 gtcgcttcac caatgttgct acttctgggg cgggacgccg tcgtggccct aactccaacg 420  
 ctgcataaga gaatggctgt atcttgatga atgtatgggt atataatcaa ttttaatacat 480  
 tcgactntat gaagttttct gttattcaag ataaatcttt ttgttgaaaa aaaaaccaag 540  
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<210> 80



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<211> 424  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 80  
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 cgatttggca attcgggatg gagttccata tccacctagg cctgcaatta ataatgttcc 180  
 tccatacctg aatatgttga ctggaacggt ttctgtacca aatgtaaatc agtacacggg 240  
 tgcaataggc cttatcgac cagcaaatcc tgtttatact tattatagct ataaatgcta 300  
 ttttccgtat agaaattatc gaggtacac actgacggat gcttactggt acgaccgtta 360  
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 ctac 424

<210> 81  
 <211> 89  
 <212> DNA  
 <213> Meloidogyne incognita

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 caacanatta cgcgccattc ttgaccca 89

<210> 82  
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 <212> DNA  
 <213> Meloidogyne incognita

<400> 82  
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<210> 83  
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 <213> Meloidogyne incognita

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 ccagtac 67

<210> 84  
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 <212> DNA  
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<400> 84  
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<210> 85  
 <211> 429  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 85  
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 tggattctct ccagtcaaaa tatgtataat ttcaaaagcg tgcttcacaa tccgaacagc 120  
 catcaacttt ttaccattgt tacgtccatg catcatcatc gaacaaacca aacgttcaac 180  
 aatcggacaa tgagcctttc gaaaacgttt gatttgatat cgaccagcac tgtgcggaac 240  
 atatttggcc gatitgtctt taacagcaat ataatccact aaagaagcat cattaacttc 300  
 gatatcgctt aaagaccatt taccaaacaa tttaatttca ggaaaatcaa ttgtagtcac 360  
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accatctcc

429

<210> 86  
<211> 435  
<212> DNA  
<213> Meloidogyne incognita

<400> 86  
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acataattgt ctctttttta ttataaaatt taaagtttaa taagttttta aacatttctg 180  
actggagttac gtgtacttag tgttttagaa aaggcaaaat tagtttggtg gtttgaagag 240  
acaaattctt ttgcacaagt agcgagaaga tatcgagcag aatttggaat ggaaccccca 300  
catatggatt tagttaaaaa attacatcaa cgttttctca atactggttc tgtttcta 360  
ggaaatactg aacattttga agttaatcca acaatggaaa catcgacatc ctcaacagag 420  
ggtgtagcag atccg 435

<210> 87  
<211> 501  
<212> DNA  
<213> Meloidogyne incognita

<400> 87  
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aaagcaattt agttttatca ataaaaattaa aaatagtcaa tgtctcgttt cactcattag 120  
atttgtggcc cttaaagagg cgttttgggt ttggttggtg tacttcagct gccttccacc 180  
aattgttcct tagccaccaa atccgtaaag agtacgtcct tggcgtttca acgcatagac 240  
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gatcacattt tcaaggaaga ctttcagaac acctcgagtc tcctcgtaaa tgagcccga 360  
aatacgtttc actccaccac gacgtgccaa tcgcccggatt gccggtttgg tgataccttg 420  
gatgatatca cgcaagactt ttcgggtggcg cttagcgcct ccctttccaa gtccctttcc 480  
gccttttact cgtccggaca t 501

<210> 88  
<211> 270  
<212> DNA  
<213> Meloidogyne incognita

<400> 88  
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ttagaataat aatggaatat ataaaaataa attggatgat ttaataaaaa aaaaaaagag 120  
agaactagtc tcgagttttt tttttttttt tttttaanaa ttaacaattt atctcatttt 180  
cctcttccat gaaaattaac aaaaagacga caacttaatc ccataattaa catcattttt 240  
aagcttcagt cggcatgctt cgaataatgt 270

<210> 89  
<211> 286  
<212> DNA  
<213> Meloidogyne incognita

<400> 89  
caagcggttc ccaactcaat gttgttgcca tgatactcgt gaacaccagt tctcgccaac 60  
atagaatagt actcaatctc actgcgtcta aggccttgag tattattcga aataataaca 120  
agtttagcct ttccagaacg aagagtcctt aacgtctgct ttagcccaa acaatacttg 180  
cccgatttgg taaccatggc gagacgagca ttgatatttt ctgtggactt tttctgtttt 240  
ccaacaacca ttgtaacgca aaattaaaaa ctctttttta acaaat 286

<210> 90  
<211> 391  
<212> DNA  
<213> Meloidogyne incognita

&lt;400&gt; 90

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tcctaggcct	gcaattaaca	atgttcctcc	atacctgaat	atgttgactc	gaacattttc	180
tgtaccaa	gtaaatcagt	acacgggtgc	aataggctct	tatcgaccag	taaatcctgt	240
ctatacttat	tatagctata	aatgctat	tccgtataga	aactatcgag	gctacacatt	300
gacggatgct	tattgggtacg	accgttatta	ttatttttcg	cctatataca	aacgggtcaat	360
gtttccaatt	agattccggc	actctgacta	c			391

<210> 91  
 <211> 131  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 91  
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 caacaaatta ccgcccgttc ttcgaccac gcacacgcgc atcattttca agaccttatg 120  
 attacacatc a 131

<210> 92  
 <211> 571  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 92  
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 cctcaaaaaa ttcattttatt gacgaccagc agcagggtgt tgctgctgtt gttgaccacc 180  
 acccccttgc gcttgacctt gctgttgctg tcccttcacg tcaacaggca aattgagttg 240  
 caaataatca accatctcct tagtctcttg atcaacacta atagttggat gttgagaagc 300  
 atcaagatag gaaacttctg gaacccaatt atcacgacgc tcacgctctt cttcttgcaa 360  
 tttaatagaa attccacgaa caggctcttt ttcgatacgc ttcacaaat gggaataaaa 420  
 accagcaatt tgattacgca tccgtttgct aggaataaca gcaatttcct cacaaattcg 480  
 tttgttcaca tgaaaatcat aagtcaagcg tgtataatat ttgtcaataa taacacgaga 540  
 tgctttcttg acagttttga gagaaccgat t 571

<210> 93  
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 <212> DNA  
 <213> Meloidogyne incognita

<400> 93  
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 aattgcaaag ggtgatgttt ttggaaagga aacgcccatt gttctggtta tgttgatatt 180  
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 gaattcttata gctgtcgagc cagtcacgac tgaagaggca gcgttcaaag acattgatta 300  
 tgcttttctt gttggtgcaa tgcctcgaaa ggaagggaatg gaacgaaagg atttacttgc 360  
 tgctaattgtg aaaatatatta aatcgcaagg attggctcta gcaaaatatt caaagccaac 420  
 tgtaaggtt ctggttggtg gaaatccagc aaatacaaat gcttttattt gtgcaaaaata 480  
 cgcagcagat aaaattccag caaagaatgt cagcgcctatg actcgtcttg accataaccg 540  
 tgcaattgcc caaatagctg ctcgttggtg gggttgactgt ggatctgtga agaaagttat 600  
 aatttgggga aatcattcaa gtacccaatt tcctgatgtt aaacatgcta aagtaattaa 660  
 aggtggcacg g 671

<210> 94  
 <211> 289  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 94  
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 agctgttatt atcgaaaaac gcaaaactgtc cagcgcaatg tcggcagcaa aggcggcatg 120  
 tgatcacatt catgattggc acttttggaa aaaagatggc gattgggttt ctatggccgt 180  
 tccttccgat ggttcttatg gaattccgga aggtttgatc ttctcatttc caattacaat 240

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tgatgcanaa acgcgtgact ggaaaattgt acaaagatta gaactcgat 289

<210> 95  
 <211> 262  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 95  
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 acgcgtgctg gttactggag cagctgggtca gattgggtat tctttgggta ttcaaattgc 120  
 aaagggagat gttttcggga aagaaacgcc catcggtctg gtaatggttg atattcctcc 180  
 aatggccgaa gtgcttaaag gagtggaaact tgaactttac gattgtgcct tggcaaatct 240  
 tatagctgtc gagccagtca cg 262

<210> 96  
 <211> 323  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 96  
 aagacattga ctatgctttt cttgtttggtg caatgcctcg aaaagaagga atggaacgaa 60  
 aggattttact tgctgctaatt gtaaaaatat ttaaatcgca aggactggct ctagcgaaat 120  
 attcaaagcc aactgttaag gttctggttg ttggaaatcc agcagataca aatgctttta 180  
 tttgtgcaaa atatgcagca gaaaaaattc cgacaaagaa tttcagcgct atgactcgtc 240  
 ttgaccataa ccgtgcaatt gcccaaatac ctgctcggtg tgtgggttgac tgtgggtctg 300  
 tcaagatagt tataatgtgg gga 323

<210> 97  
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 <212> DNA  
 <213> Meloidogyne incognita

<400> 97  
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 cacttttcctg aactggagggt tcataagcat ctggacgact ttcaataact tctccacttg 180  
 ctgtagttat agcaacttgt ccaccaccac ttccagcacc ctctccatgc atatccaaaa 240  
 gttttccaag ttcaaatttt ggttttttca aaatttttac ttttcgaata taaacgtctt 300  
 gaagtggata gaaataagaa caagactttt caatgtcttt tccaatagaa tcaggaatta 360  
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 ttggagcttt ctttgtgaag ccaatacgaag acaatcgaag caaataacca tcagttgttt 540  
 tgacagcaac atttgcttca attaaagtat gccacttttt gacaatagaa caaagcttgt 600  
 ctgagataaa agtcattcca tggaaattgg tcaaacaacac tttgccttga acctcttcac 660  
 aaataagtcg aaatttgcca aagtcagctt cgggtgttgtt cagatcacca agagaaa 717

<210> 98  
 <211> 758  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 98  
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 aaaaagaagg atggcttcga tgccaaaaag tttgcgattg atttggcttc tggaggaact 180  
 gccgctgcgg tttctaagac ggctgtggcg cctattgaac gtgtcaagtt gttgctacag 240  
 gttcaagacg cttctcagca catcgctgcc gataaacgct ataaaggaaat aattgatgtg 300  
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 gtgatccgtt actttccaac gcaagctctc aactttgcgt tcaaggacac ttacaagagg 420  
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 ctgtgatacg tatacaaac tctcttcaat tggagattca atgttgagtg gagatgctga 600  
 tagtaatcct ctgttacaat cacttaacaa ctcaatcaat tccaatgcca ctgctcagaa 660  
 ttataactcc tcaacaattg gccgaagcta aaaactacgt ttcaacatgc tacagctact 720

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tcaaaatcga aacagattgt tttaaacgtt tgaaattt 758

<210> 99  
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<212> DNA  
<213> Meloidogyne incognita

<400> 99  
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tgccatttct cgcaggttat tggcacttca cacatttgta ccaataacaa cgttaccgtt 120  
tataatcaaa ctgttcctca aagttatgcc catt 154

<210> 100  
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<212> DNA  
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<400> 100  
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tcgta 125

<210> 101  
<211> 219  
<212> DNA  
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<400> 101  
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tttggactgg ttgagtaacc aattgaagaa tcaaggttaa atgagtctaa ataaaaatgg 180  
agagggggaaa gaggagaggt taatttttta aggaaaaaa 219

<210> 102  
<211> 473  
<212> DNA  
<213> Meloidogyne incognita

<400> 102  
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atgggggaaa aaataggagc aagccaaaaa gccaaaaaaa aatttttttt ttaaattgatt 120  
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<210> 103  
<211> 114  
<212> DNA  
<213> Meloidogyne incognita

<400> 103  
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<210> 104  
<211> 255  
<212> DNA  
<213> Meloidogyne incognita

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<400> 104  
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 acgtaaaaaca cagtggaaat ttgacgatcg agcaaaattat caacattgca cggcaaatgc 120  
 gacctcgttc aatggcgaaa aaaattggaa gggactgtta aggaaattct tggcactgca 180  
 caatctgttg ggtgtactgt tgatggacaa catccacatg atattgttga tgcaatccga 240  
 agtgggaaaaa ttgaa 255

<210> 105  
 <211> 571  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 105  
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 taacacacat ttttaattcc ttaatactcc aaaaaacttc tcttctttat tccctcttat 120  
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 cttatgaatg tcaaaaacag cagcaacaac ccccagcaga accttgtgga ctttctttgg 480  
 aagttcatca atctggtcat tcaacaacaa cccttccatc tccatgttct ttattacccc 540  
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<210> 106  
 <211> 235  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 106  
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 aatttttagca gcattagccc caactacttt agctgctaataaaaattgttt atgaggatgg 120  
 agatagtgat ggacttgata tggctaaaag tatttttaaat tgaataaagg aaaaagaagc 180  
 attttaaaga aaatttagatg gaaatgctga agaaagaaaa aaattattta ttttt 235

<210> 107  
 <211> 702  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 107  
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 ctctcaaatg gaggagtatc aacgtttctt cgatatgttt gaccgtggaa agaattggcta 180  
 tattatggct actcaaattg gggtaattat gaatgctatg gaacaagatt ttgatgaaaa 240  
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 aacactcaaa ggattacttc acgaaatcgc cccagacctc agcgataaag acttggatgc 480  
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 tattttgcca ttaaattttg gaaagtgcga aaaattgcct ttctgagaat ttttattttt 660  
 aacgtctaaa taatgaataa aatggatata aaaaaaaaaa aa 702

<210> 108  
 <211> 423  
 <212> DNA  
 <213> Meloidogyne incognita

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 agaaagaaaa tgccaaagga gatgaagaac ttgttgaaga aaaaagttca aaaatatcaa 180  
 ctctccatt tgctcgtcaca ttttctttca ttattccatt tgttgtaagc tcagtaactg 240

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cccccaattgt tgtttagtagc catggagaga aagcactttc cccattcgaa aatgttgaac 300
caaattgggtc aaattgttgc tgttgttgac ctcgaagtgc gttagaaaca gaacgaaata 360
aattatgagg ttgttgttgc tcctgacggt tttgattgtc tggagctggg tgaggatcac 420
caa                                         423

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<210> 109  
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 <212> DNA  
 <213> *Meloidogyne incognita*

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tgaaattgaa cttgtcgatc catcgttaaa gggcaaaatt attattaaag caaacaaaaa 240
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ggaagattta gatgaactaa aattggatga tggcgttgaa aatgtgCGaa agataataac 420
gaaattcaga taaaaataac aaagaaaagt ttataataaa agctgagttt gccgatatcg 480
acccaaaaat tgttgatctt ttacagaaa ttgggtcaagt tttaaagaaa tatagaagtg 540
gacgtattcc caaagctttt aaagttattc caactttggt tgattgggag aaaattatcg 600
aattaactcg cccagatgat tggtcggcag ctgcaatggt acatgctacc aaaatatttg 660
cttcaactgc tacccctact caatgccaaa ggttttataa tttgattttg ttgccacgta 720
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caatttttct cttcgagaag ctgttgttct tgcttctatg cttcgtaaag cctccatccc 900
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<210> 110  
 <211> 476  
 <212> DNA  
 <213> *Meloidogyne incognita*

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aaattgagga gtatcaacgt ttcttcgata tgtttgaccg tggaaagaat ggctatatata 180
tgggccactca aattggggta attatgaatg ctatggaaca agattttgat gaaaaaactc 240
ttcgaaaaatt aatccgaaaa ttcgacgcag acggcagcgg caaaatcgaa ttcgacgaat 300
tctgcgctct ggtatacact gtggcgaaata ctgtagataa ggacactttg cggaaagaat 360
tgagagaagc ttttcgtctc ttcgacaagg agggtaatgg ttacatctct cgtccaacac 420
tcaaaggatt actccacgaa atcgccccag acctcagcga taaagacttg gatgcc 476

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<210> 111  
 <211> 189  
 <212> DNA  
 <213> *Meloidogyne incognita*

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<400> 111
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tgattgaaat ttaatttaga gatgaataaa aaattaacta aaatattttg ccataaaaatt 120
ttggaaaagt ccaaaaattg cctttttgag aattttttatt tttaacgtct aaataatgaa 180
taaattggat                                         189

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<210> 112  
 <211> 164  
 <212> DNA  
 <213> *Meloidogyne incognita*

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<400> 112
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aaaaacaaca tttttaaatc aaatgacaga catatatatt caataacgat gtgtggattt 120
tctttttttt taaataatta acatctttaag cctgctattt cttc                                         164

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<210> 113  
 <211> 539  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 113  
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 taacaccaac agccacagtt tgacgcatgt cacgaacggc gaagcgtcca agaggagcgt 120  
 agtcagtaaa agcctcaaca cacattggct tggittggaat taagtcgaca ataccagcat 180  
 ctccagtcctt caaagccttt ggattgtctt caaccttctt tccagttcga cggtcgacct 240  
 tctctttaag ctacgcgaac ttgcaagcaa tgtgagcagt gtgacagtca agaacaggcg 300  
 tgtagccagc agcaatctgc ccaggatggg tcatgatgat aacctgagca gtgaattgct 360  
 tggctctcct tgctgggtca ttcataagat cagaagtgc tgaaccacgt cggatgtcct 420  
 tgacagagat gttcttaacg ttaaatccaa cattgtcttc aggaacagct tcaggggagag 480  
 actcgtgggtg catctcaaca gatttaactt cagtagaaat tccttcagga gcaaaggta 539

<210> 114  
 <211> 314  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 114  
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 cggagaatct tgaaagagta aggaaatgtc cagttttggg tgttgggtgct ggtgggcttg 180  
 gatgtgaaat ttgaaaaat ttggccttat caggatttca aaatattgaa gttattgata 240  
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 gcttatacaa agca 314

<210> 115  
 <211> 200  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 115  
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 gaagatgggt aaacgccaca gtgtgttctt tggtcgaatt tgacgaagaa aacaagccac 180  
 ggccaggcac aattattcca 200

<210> 116  
 <211> 471  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 116  
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 gagccattgt tgattgtagg aaaggacaaa tttgctggaa tggatattcg catccgtgtc 180  
 aaagggtggt gtcattgtgc acaaatttat gcaattcgac agtcaattgc taaagttttg 240  
 gtggcctatt accagaaaaa cgtggatgag caaagcaaga aagaattgaa ggatcaactt 300  
 gttgcttatg atcgtaattt gcttgttgcc gatccgagac gtcacgagcc aaagaagttt 360  
 ggaggacctg gtgctcgtgc tcgttatcag aaatcttatc gttaagaagt atgaaattat 420  
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<210> 117  
 <211> 593  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 117  
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&lt;210&gt; 118

&lt;211&gt; 576

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 118

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tgagggggaa	gtaaaatgaa	agaagggaga	gagatatgaa	ttggagggtt	ttttgttaaa	180
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ttgattgggtg	acgccgacga	ggaagtgttc	tttctctcgg	atagcaaaga	ctcgcccaat	480
atttttcagcc	tttgtgaaga	aagtgcctgt	ggggacgtaa	gcacgtctat	gttggtgttg	540
agcgcttctt	aatccagcag	aaaagcattg	aatacgc			576

&lt;210&gt; 119

&lt;211&gt; 559

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 119

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acgtcagtaa	cccaactatt	gccgtgggca	tcgattgtta	gtccatgagg	catgtaaaac	360
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gtggttgaaa	tgatgcccg	ggatctgttt	aggtggttgt	tctcatcaaa	cgaaaattca	480
tcccaaaactc	tgtcagatcg	gtgaaaaaga	acaagtgcgat	tcaatggatc	caatgcaata	540
cccggagctt	gcccataat					559

&lt;210&gt; 120

&lt;211&gt; 366

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 120

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tagtggtgct	agcgggtgtg	gtgctgctgt	tatgaataag	caagcaagta	aatacaatga	120
agttgaagga	gaactccttc	tttaattggat	taagaaagtg	acaggcgaaa	atattgctat	180
aaacgggaact	aggggaaatt	ttgtgaaaca	attgaaagat	ggaaactctgc	tctgcaaat	240
tgctaacaaa	attgtgccaa	attcaatcac	aaaggcacag	gcaaaaccga	acagcacatt	300
ccaatatatg	agcaatttgg	agctgttctt	aacattttatt	tcaagccaag	gagtccttag	360
ggagga						366

&lt;210&gt; 121

&lt;211&gt; 661

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 121

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ggtagctggag	accaagtgcg	ccttcgtgtt	taaagatggg	aaattgaaag	aattttgggt	180
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cgctactttc	atcattttccg	atcgaccttt	gttgttttct	aagttcgttg	gccaaagaaa	360
ggatatgtaa	aattgaatta	tgaataaaaa	taaatcactc	aatcagaggg	attgttagtc	420
tctcactttc	tcctctttac	ccattggcta	accagcttta	aggatttttt	ccataagttc	480
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taaaaaaaaa	ttttttttat	tttttttcca	taatgctatc	tatatatttt	gcttttaatc	600
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<210> 122  
 <211> 173  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 122						
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gggaaattgc	tcagtcaatt	tgggaagcct	gactccagtg	aaacacccaa	aaatggagaa	120
ttccttgtag	cacactctct	aaccctcatt	gaagatctca	acttactttg	tgt	173

<210> 123  
 <211> 584  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 123						
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gagaaagaac	acttcctcgt	cggcgtcacc	aatcaagatc	agggcagtc	attagaatcc	180
caagttttcg	taatggatat	gaacacagga	agggctaata	gctttgctaa	gggtctagaa	240
aacgcccattg	cccttgcaat	cagcgataat	ggagatatatt	atgtttcaca	aatagaaccc	300
aaccaaattg	taaaatttag	tatttcgaca	aacgaaaatt	gagaaaaaaa	aaaaaaaagc	360
tcagaaacgg	gaagaatttt	caagaaaaaa	tttttttacc	aaacaaaaaa	cctccaattc	420
atatctctcc	cttctttcat	ttttccttcc	ccttctcccc	aaaaattaca	aaaaatttta	480
ttgtgcacaa	aaaaatgggc	gggcggggcga	atggctgggc	aaaggatggc	gataaatctt	540
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<210> 124  
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 <212> DNA  
 <213> Meloidogyne incognita

<400> 124						
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ctcttctcaa	gaagtacttc	acgaagggaag	ttatggacca	gtgtaaagg	ctcaaaacta	180
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<210> 125  
 <211> 1013  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 125

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gttttgaatc	aaataattaa	attttaaatt	atttaaacag	ctacacgagg	cctcagcctc	180
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<210> 126  
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 <212> DNA  
 <213> Meloidogyne incognita

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 cttaccaaat gggatcaaat 80

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 <211> 585  
 <212> DNA  
 <213> Meloidogyne incognita

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 aagcgggcat gacagggtttt ggaactccaa ggaacacaa acacgataat atacgaggcg gactctggcg 180  
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 aacatttgaa gcgtatttgg gagttggaat acccagagga ggctgaaatt tcgttggatc 360  
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<210> 128  
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 <212> DNA  
 <213> Meloidogyne incognita

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<210> 129  
 <211> 175  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 129  
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ttccacaact cttcaatcaa gtttcctttg ccgcatcgtg tgaatgacaa acgtc 175

&lt;210&gt; 130

&lt;211&gt; 599

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 130

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 aaagggttcac cttctaagta aggattgtag cggctgtatg attgatgttg cttttgttgg 120  
 ggagcaatag aacgcttgcg tcgccgaggc tcctcagccc tagtaacgtg aaatttcttt 180  
 gcaatcatcg atttgtgtag tccatttttg gctaagacct gttctaagtc ttgttcatat 240  
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 ctttcaccgg cacctcctgt cataaattta tgtccaaacg caatgggccc ggaagcactt 540  
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&lt;210&gt; 131

&lt;211&gt; 466

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 131

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&lt;210&gt; 132

&lt;211&gt; 266

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 132

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&lt;210&gt; 133

&lt;211&gt; 308

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 133

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&lt;210&gt; 134

&lt;211&gt; 335

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

## AKK110P1

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